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(54) **Title**: SAM-6 VARIANTS, TARGET AND METHODS OF USE

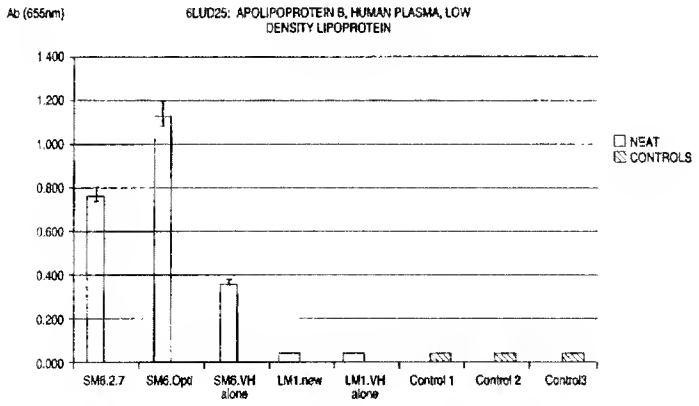

FIG. 29

(57) **Abstract**: The application relates to variants and functional fragments of the SAM-6 antibody that bind to one or more of glucose regulated protein 78 (Grp78), deglycosylated Grp78, apolipoprotein B100 (apoB100), and glycosylated or deglycosylated lipoproteins VLDL and LDL. Use of the variants and functional fragments in the treatment and diagnosis of neoplasia, tumour, cancer and disorders or diseases associated with excessive levels of LDL or oxidised LDL is also described.

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SAM-6 Variants, Target and Methods of Use

Related Applications

This application claims priority to U.S. Provisional Application Serial No. 61/151,149, filed February 9, 2009, which is incorporated by reference herein in its entirety.

Field of the Invention

The invention relates to antibody variants, and target peptide that binds to an antibody, known as SAM-6. The glycoprotein, denoted SAM-6 Receptor (SAM-6/R) or SAM-6/R Target has apparent structural homologyy with Grp78 (glucose regulated protein 78) and apoB100 polypeptide sequence.

Introduction

There is a need for compounds and methods of treating undesirable or aberrant cell proliferation, such as cellular hyperproliferative disorders, including neoplasias, tumors and cancers. The invention addresses this need and provides related benefits.

Summary

The invention provides isolated and purified glycoproteins denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. In one embodiment, a SAM-6/R glycoprotein has an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from Grp78, and an antibody denoted SAM-6 specifically binds to the SAM-6/R glycoprotein. In another embodiment, a SAM-6/R glycoprotein has an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from a carbohydrate moiety of Grp78, and polypeptide sequence homology to Grp78 as set forth in SEQ ID NO:1 (*e.g.*, at least 60%, 70%, 80%, 90%, 95% or more identity).

In various aspects, isolated and purified glycoproteins denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein include a sequence of about 655 amino acids, have a transmembrane domain of about 17 amino acids, have an extracellular domain of about 220 amino acids, or have an intracellular domain of about 411 amino acids. In various additional aspects, SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein is characterized as being expressed on or secreted by a neoplastic, cancer or tumor cell, for example, a pancreas carcinoma cell line or lung carcinoma cell line denoted respectively as BXPC-3 (ATCC Deposit No. CRL-1687) and A549 (DSMZ Deposit No. CCL185).

In another aspect, a cell surface or secreted Grp78 may be in association with another antigen, for example, function as a chaperone. Human plaminogen Kringle 5 (an inhibitor of endothelial cell growth) or another antigen.

In a further aspect, the linked carbohydrate moiety of isolated and purified glycoproteins denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein is linked to an asparagine, serine or threonine residue (e.g. of SEQ ID NO:1). In a further aspect, the SAM-6 antibody binds to a portion of the glycoprotein that includes the N- or O-linked carbohydrate moiety, or binds to the N- or O-linked carbohydrate moiety. In additional aspects, treatment of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein with a glycosidase enzyme (*e.g.*, an O-glycosidase or an N-glycosidase, such as endoglycosidase H or endoglycosidase F) reduces the apparent molecular weight of the glycoprotein by about 1-5 kilodaltons (kDa), or reduces binding of SAM-6 antibody to the SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. In a still further aspect, the N- or O-linked carbohydrate moiety includes one or more of galactose, acetylgalactose, mannose, fucose, glucose, acetylglucose, sialic acid, N-acetylgalactosamine, or N-acetylglucosamine.

The invention also provides subsequences of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. In one embodiment, a subsequence includes a portion of the glycoprotein with an N- or O-linked carbohydrate moiety.

The invention further provides nucleic acid sequences that encode glycoprotein SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. In one embodiment, a nucleic acid sequence is at least 75-90%, or more, complementary or homologous to a nucleic acid sequence that encodes SEQ ID NO:1, or a subsequence thereof. In another embodiment, a nucleic acid sequence encodes a SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from a carbohydrate moiety of Grp78. In particular aspects a nucleic acid encodes a subsequence of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. In other aspects, a nucleic acid sequence has a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000 nucleotides. In additional aspects, a nucleic acid sequence specifically hybridizes to a nucleic acid that encodes SEQ ID NO:1, or a subsequence thereof, or specifically hybridizes to a nucleic acid sequence complementary to a nucleic acid that encodes SEQ ID NO:1, or a subsequence thereof. In further aspects, a nucleic acid is an antisense polynucleotide, a small interfering RNA, or a ribozyme nucleic acid that specifically hybridizes to a nucleic acid sequence encoding SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein and reduces expression of the SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. Antisense polynucleotides, small interfering RNA, and ribozyme polynucleotides can have a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-

1000, 1000-2000 nucleotides, and be at least 90% complementary or homologous to a nucleic acid sequence that encodes SEQ ID NO:1, or a subsequence thereof. In still further aspects, nucleic acid sequence can include an expression control sequence or a vector (*e.g.*, a viral, bacterial, fungal or mammalian vector).

The invention additionally provides host cells transformed with nucleic acid that encodes SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein or a subsequence thereof. Host cells include eukaryotic (*e.g.*, a hyperproliferative cell, immortalized cell, neoplastic cell, tumor cell or cancer cell) and non-eukaryotic cells, which can be stably or transiently transformed with the nucleic acid or vector.

The invention moreover provides isolated and purified polyclonal and monoclonal antibodies that specifically bind to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a subsequence thereof. In one embodiment, an antibody specifically binds to an epitope or sequence of one or more of a SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a subsequence thereof. In another embodiment, an antibody does not bind to an epitope comprising an N- or O-linked carbohydrate moiety. In a further embodiment, an antibody binds to one or more of apoB100 and Grp78. In still another embodiment, an antibody binds to one or more of LDL (*e.g.*, oxidized LDL, "oxLDL"), VLDL, glycosylated or deglycosylated forms.

In an additional embodiment, an antibody competes for binding of SAM-6 antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18), to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a subsequence thereof, or inhibits or blocks binding of SAM-6 antibody (*e.g.*, at least 50% of the binding of SAM-6 antibody) to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a subsequence thereof (*e.g.*, as determined in an ELISA assay), or competes for binding of SAM-6 antibody to one or more of Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or inhibits or blocks binding of SAM-6 antibody (*e.g.*, at least 50% of the binding of SAM-6 antibody) to one or more of Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms (*e.g.*, as determined in an ELISA assay). In still additional embodiments, an antibody binds to cells expressing SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein (*e.g.*, a neoplastic, cancer or tumor cell or cell line such as BXPC-3 or A549 cells) and stimulates or induces cell death, lysis or apoptosis, or activation of a caspase (e.g. caspase-3, capase-7, caspase-8 or caspase-9) *in vitro* or *in vivo*. In still another embodiment, treatment of

SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein with an O-glycosidase reduces binding of the antibody to the SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, or to LDL or oxLDL.

In still additional embodiments, the antibody has a binding affinity for one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, within about 1-5000 fold of the binding affinity of SAM-6, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18), or a binding affinity for one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, within about KD 10^{-5} M to about KD 10^{-13} M of SAM-6. In yet further embodiments, the antibody has a binding affinity for Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, within about 1-5000 fold of the binding affinity of SAM-6, or a binding affinity for Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, within about KD 10^{-5} M to about KD 10^{-13} M of SAM-6 (e.g., as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

Antibodies of the invention include those distinct from SAM-6 antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18). In one embodiment, an antibody does not have heavy and light chain sequences identical to heavy and light chain sequences of SAM-6. In another embodiment, an antibody does not have heavy or light chain variable sequences identical to heavy or light chain variable sequences of SAM-6. In a further embodiment, an antibody does not have 90%-95%, 96%, 97%, 98%, 99%, or more identity to heavy or light chain variable region sequence of SAM-6 antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18). In a particular embodiment, an antibody that is distinct from SAM-6 antibody has a heavy chain sequence with 100% identity to a heavy chain variable region sequence or a CDR (CDR3; ARDRLAVAGRPFDY; SEQ ID NO:17) within a heavy chain variable region amino acid sequence set forth as SEQ ID NO: 18, or a CDR3 of antibody heavy or light chain variable region represented by antibody produced by hybridoma deposited as DSM ACC2903, or a light chain variable region sequence (SEQ ID NO:13) or a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

In still further embodiments, the antibody heavy chain variable region (V_H) alone can bind to a target antigen. In particular aspects, a heavy chain variable region (V_H) alone can bind to one or more of a glycosylated or deglycosylated SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, a glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL, glycosylated or deglycosylated VLDL or oxidized glycosylated or deglycosylated LDL.

Antibodies of the invention include IgG, IgA, IgM, IgE and IgD. In various aspects, an IgG is an IgG_1, IgG_2, IgG_3, or IgG_4.

Antibodies of the invention also include antibody subsequences of the antibodies set forth herein, such as subsequences that bind to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a subsequence (*e.g.*, immunogenic fragment) thereof. In various aspects, a subsequence is an Fab, Fab', $F(ab')_2$, Fv, Fd, single-chain Fv (scFv), disulfide-linked Fvs (sdFv), V_L, V_H, trispecific (Fab_3), bispecific (Fab_2), diabody ($(V_L\text{-}V_H)_2$ or $(V_H\text{-}V_L)_2$), triabody (trivalent), tetrabody (tetravalent), minibody ($(scFv\text{-}C_H3)_2$), bispecific single-chain Fv (Bis-scFv), IgGdeltaCH2, scFv-Fc or $(scFv)_2$-Fc.

The invention also provides SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein and subsequences thereof, antibodies and subsequences that bind to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, that include a heterologous domain. In one embodiment, a heterologous domain includes a detectable label, tag or cytotoxic agent. In particular aspects, a detectable label or tag is an enzyme, enzyme substrate, ligand, receptor, radionuclide, a T7-, His-, myc-, HA- or FLAG-tag, electron-dense reagent, energy transfer molecule, paramagnetic label, fluorophore, chromophore, chemi-luminescent agent, or a bio-luminescent agent.

The invention still further provides kits. In one embodiment, a kit includes an antibody that binds to one or more SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, and instructions for detecting one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms. In another embodiment, a kit includes an antibody that binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, and instructions for treating a condition treatable with an antibody that binds to bind to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms. In additional embodiments, a kit also includes an anti-cell proliferative or immune enhancing treatment or therapeutic agent, or an anti-neoplastic, anti-cancer or anti-tumor agent, or an article of

manufacture (*e.g.*, for delivering the antibody, anti-cell proliferative or immune enhancing treatment or therapy into a subject locally, regionally or systemically). In particular aspects, the instructions are for treating undesirable cell proliferation or hyperproliferation or treating a neoplasia, tumor or cancer. In additional aspects, the instructions are on a label or packaging insert.

The invention yet additionally provides pharmaceutical compositions. In one embodiment, a composition includes SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL, and a pharmaceutically acceptable carrier or excipient. In another embodiment, a composition includes an antibody or subsequence thereof that specifically binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, and a pharmaceutically acceptable carrier or excipient.

The invention still further provides methods for treating a disorder in a subject in need of treatment. In one embodiment, a method includes administering SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein or an antibody that specifically binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, in an amount effective to treat a cellular hyperproliferative disorder in a subject (*e.g.*, a stage I, II, III, IV or V, metastatic or non-metastatic, solid or liquid neoplasia, tumor or cancer). In various aspects, a cellular hyperproliferative disorder affects or is present at least in part in brain, head or neck, breast, esophagus, mouth, nasopharynx, nose or sinuses, stomach, duodenum, ileum, jejunum, lung, liver, pancreas, kidney, adrenal gland, thyroid, bladder, colon, rectum, prostate, uterus, cervix, ovary, bone marrow, lymph, blood, bone, testes, skin or muscle, or hematopoetic system. In additional aspects, a cellular hyperproliferative disorder includes a neoplasia, tumor or cancer that affects or is at least in part present in breast, lung, thyroid, head and neck, nasopharynx, nose or sinuses, brain, spine, adrenal gland, thyroid, lymph, gastrointestinal tract, mouth, esophagus, stomach, duodenum, ileum, jejunum, small intestine, colon, rectum, genito-urinary tract, uterus, ovary, cervix, bladder, testicle, penis, prostate, kidney, pancreas, adrenal gland, liver, bone, bone marrow, lymph, blood, muscle, skin or is hematopoetic. In particular aspects, a neoplasia, tumor or cancer is a sarcoma, carcinoma, adenocarcinoma, melanoma, myeloma, blastoma, glioma, lymphoma leukemia. In additional particular aspects, a neoplasia, tumor or cancer is a lung adenocarcinoma, lung carcinoma, diffuse or interstitial gastric carcinoma, colon adenocarcinoma, prostate adenocarcinoma, esophagus carcinoma, breast carcinoma, pancreas adenocarcinoma, ovarian adenocarcinoma, or uterine adenocarcinoma. In further particular aspects, a neoplasia, tumor or cancer is progressively worsening or is in remission. In still additional aspects, treatment results in alleviating or

ameliorating one or more adverse physical symptoms associated with a cellular hyperproliferative disorder, or a neoplasia, tumor or cancer, or reduces or decreases neoplasia; tumor or cancer volume, inhibits or prevents an increase in neoplasia, tumor or cancer volume, inhibits neoplasia, tumor or cancer progression or worsening, stimulates neoplasia, tumor or cancer cell lysis or apoptosis, or inhibits, reduces or decreases neoplasia, tumor or cancer proliferation or metastasis, or prolongs or extends lifespan of the subject, or improves the quality of life of the subject.

Methods include administration to the subject locally, regionally, or systemically. Exemplary subjects (*e.g.*, mammals such as humans) include candidates for, and those undergoing, or having undergone an anti-cell proliferative or anti-cellular hyperproliferative disorder (*e.g.*, anti-neoplastic, anti-tumor, anti-cancer or anti-metastasis) or immune-enhancing treatment or therapy.

The invention yet also provides combined methods for treating a disorder in a subject in need of treatment. In one embodiment, a method includes administering SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein or an antibody that specifically binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, and an anti-cell proliferative or immune-enhancing treatment or therapy to a subject (*e.g.*, prior to, substantially contemporaneously with or following each other). In various aspects, an anti-cell proliferative or immune-enhancing treatment or therapy includes surgical resection, radiotherapy, radiation therapy, chemotherapy, immunotherapy, hyperthermia, an alkylating agent, anti-metabolite, plant extract, plant alkaloid, nitrosourea, hormone, nucleoside or nucleotide analogue, a lymphocyte, plasma cell, macrophage, dendritic cell, NK cell or B-cell, an antibody, a cell growth factor, a cell survival factor, a cell differentiative factor, a cytokine or a chemokine.

The invention still additionally provides methods for treating a disorder or disease associated with or caused by undesirable or excessive VLDL, LDL or oxLDL levels. In one embodiment, a method includes administering to a subject an antibody that specifically binds to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein in an amount effective to treat the disorder or disease associated with or caused by undesirable or excessive VLDL, LDL or oxLDL levels in the subject. In various aspects, a disorder or disease associated with or caused by undesirable or excessive VLDL, LDL or oxLDL levels is hyperlipidemia, hypercholesterolemia, arteriosclerosis, cardiovascular disease, coronary heart disease (CHD), stroke, glomerulonecrosis, high blood pressure or diabetes.

The invention provides methods for detecting or screening for SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms. In one embodiment, a method includes contacting a biological material or

sample with an antibody that specifically binds to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL under conditions allowing binding of the antibody to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, and assaying for binding of the antibody to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms. The binding of the antibody to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, detects the presence of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms. In one aspect, SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, is present on a cell or tissue. In another aspect, the biological material or sample is obtained from a mammalian subject.

The invention further provides methods for diagnosing a subject having or at increased risk of having a cellular hyperproliferative disorder (*e.g.*, neoplasia, tumor or cancer, or metastasis). In one embodiment, a method includes providing a biological material or sample from a subject, contacting the biological material or sample with an antibody that specifically binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, under conditions allowing binding of the antibody to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, and assaying for binding of the antibody to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. The binding of the antibody to one or more of the foregoing diagnoses the subject as having or at increased risk of having a neoplasia, tumor or cancer, or metastasis. In one aspect, the biological material or sample is obtained from a human. In additional aspects, the biological material or sample comprises a biopsy, such as a lung, pancreas, stomach, breast, esophageal, ovarian or uterine biopsy. In further aspects, the biological material or sample comprises serum, plasma, urine, saliva, menstruate, or feces.

The invention additionally provides methods for producing an antibody that specifically binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms. In one embodiment, a method includes administering SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms or a fragment thereof, to an animal, screening the animal for expression of an antibody that binds to one or more of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL

(*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or the fragment thereof, selecting an animal that produces an antibody that binds to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or the fragment thereof, and isolating the antibody from the selected animal. In another embodiment, a method includes administering SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a fragment thereof to an animal capable of expressing a human immunoglobulin, isolating spleen cells from an animal that produces antibody that binds to SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or the fragment thereof, fusing the spleen cells with a myeloma cell to produce a hybridoma, and screening the hybridoma for expression of a human antibody that binds to the polypeptide or the fragment thereof. In particular aspects, the fragment of SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein includes a portion of the polypeptide sequence with an N- or O-linked carbohydrate moiety, for example an antigen, epitope or structure of Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL to which a SAM-6 antibody binds.

Description of Drawings

Figure 1: SAM-6 lipoptotic pathway.

Figure 2: SAM-6 target.

Figure 3: Representative western blot of SAM-6 and unrelated IgM control on membrane extracts.

Figure 4: Chromatogram after size exclusion chromatography.

Figure 5: Western blot analysis of SAM-6 positive fractions after size exclusion chromatography.

Figure 6: Coomassie staining of SDS-Page gel after size exclusion chromatography.

Figure 7: Chromatogram after ion exchange chromatography.

Figure 8: Western blot analysis of SAM-6 positive fractions after ion exchange chromatography.

Figure 9: Coomassie Blue Staining after ion exchange chromatography.

Figure 10: Peptide mass map of isolated 80kDa protein obtained by MALDI mass spectrometry analysis.

Figure 11: Alignment of experimentally determined peptide sequences assigned to human Grp78 and the protein sequence of human Grp78/BiP [NP_005338].

Figure 12: FACS analysis of SAM-6 binding on Grp78-siRNA-transfected BXPC-3 cells.

Figure 13: Analysis of SAM-6 binding on Grp78-siRNA-transfected BXPC-3 cells. Percentages of cells positive for SAM-6surface antigens and controls (Grp78 and CD55) are shown.

Figure 14: Cell Death ELISA with Grp78-siRNA-transfected BXPC-3 cells.

Figure 15: Western Blot Analysis: Binding of SAM-6 antibody on membrane extracts of pancreas carcinoma cell line BXPC-3.

Figure 16: SAM-6 binding on pancreas cancer cells after treatment with glycosidases.

Figure 17: Immunoflourescence of SAM-6 endocytosis.

Figure 18: Sudan III staining of antibody-induced pancreas cancer cells (BXPC-3).

Figure 19: Analysis of SAM-6 induced apoptosis by measurement of Cytochrome C release.

Figure 20: Analysis of SAM-6 induced apoptosis by measurement of activation of caspases -8, -9, -3 and -6.

Figure 21: SAM-6 Dose dependent inhibition of stomach carcinoma tumour xenograft growth.

Figure 22: Shows a diagram of sequence coverage for the heavy chain variable region of SAM-6 antibody produced by hybridoma cell line.

Figure 23: Shows a diagram of sequence coverage for the light chain variable region of SAM-6 antibody produced by hybridoma cell line.

Figure 24: Shows an ELISA of 1.1A and 1.1B SAM-6 diabodies binding to LDL and a target antigen present in conditioned media of A549 cells.

Figure 25: Shows functional cell binding and cell death ELISA.

Figure 26: Shows an immunoprecipatation of A549 conditoned media with SAM-6 diabody. The diabody binds to target antigens with a molecular weight in the range of 60-100 kDa.

Figure 27: Shows an LDL ELISA of certain SAM-6 variants.

Figure 28: Shows binding of the SAM-6 V_H alone to Hela cells.

Figure 29: Shows an analysis of binding of SAM-6 2.7, Single-chain antibody of SAM-6 Opti (kappa light chain), and SAM-6 V_H to recombinant expressed Grp78 (glycosylated).

Detailed Description

The invention is based, at least in part, on a glycoprotein, referred to as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein. SAM-6/R glycoprotein has an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis. A non-limiting exemplary feature of SAM-6/R glycoprotein is linkage of at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety that is distinct from Grp78. Another non-limiting exemplary feature of SAM-6/R glycoprotein is that an antibody denoted SAM-6, produced by hybridoma deposited as DSM ACC2903 (deposited with the DSMZ, Braunschweig, Germany, on April 3, 2008), specifically binds to SAM-6/R glycoprotein.

In accordance with the invention, there are provided isolated or purified glycoprotein denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis. In one embodiment, a SAM-6/R glycoprotein has at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from Grp78. In another embodiment, an antibody denoted SAM-6 specifically binds to a SAM-6/R glycoprotein.

Sequence analysis of SAM-6/R glycoprotein revealed sequence identity with glucose-regulatory (or regulated) protein 78 (Grp78), also known and referred to as BiP, HspA5, Heat shock 70 kDa protein 5 and Hsce70. Human Grp78/BiP sequence is as set forth in Figure 11 (SEQ ID NO:1). Sequences of SAM-6/R glycoprotein that appear identical to Grp78/BiP sequence are indicated in bold typeface. Thus, another non-limiting exemplary feature of SAM-6/R glycoprotein is at least partial sequence homology/identity with Grp78.

In accordance with the invention, there are provided isolated or purified glycoproteins denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel

electrophoresis. In one embodiment, SAM-6/R glycoprotein has polypeptide sequence homology to Grp78 as set forth in SEQ ID NO:1.

In various non-limiting aspects, a SAM-6/R glycoprotein comprises a sequence of about 655 amino acids; has a transmembrane domain of about 17 amino acids; has an extracellular domain of about 220 amino acids; or has an intracellular domain of about 411 amino acids. In other non-limiting aspects, the SAM-6/R glycoprotein has a carbohydrate moiety linked to an amino acid, for example, an asparagine, serine or threonine residue of (*e.g.*, as in SEQ ID NO:1). Potential O-glycosylation sites of SAM-6/R glycoprotein are indicated by the underlined threonine (T) residues in SEQ ID NO:1.

As used herein the term "glycoprotein" refers to a protein, polypeptide or peptide that has at least one sugar moiety covalently linked to an amino acid comprising the protein. A "carbohydrate moiety" refers to two or more sugar residues, *e.g.*, mono-, di-, tri-saccharides, etc. The terms oligosaccharide and polysaccharide are synonymous with the term carbohydrate. A glycosylated protein is used synonymously as a glycoprotein. A deglycosylated protein refers to a protein in which one or more (or all) sugars/carbohydrate residues have been removed relative to a comparison protein.

Sugars, carbohydrates, oligosaccharides and polysaccharides are typically linked to amino acid residues by a glycosidic bond. For eukaryotes, a series of sugar additions and removals occur post-translationally to form the carbohydrate moiety of the glycoprotein. Exemplary sugars include one or more of galactose, acetylgalactose, mannose, fucose, glucose, acetylglucose, sialic acid, N-acetylgalactosamine, N-acetylglucosamine and neuramic acids. A SAM-6/R glycoprotein optionally has have one or more of such particular sugars attached via an N- or O-linkage to a serine, threonine or asparagine residue, for example.

Contact of SAM-6/R glycoprotein with a glycosidase enzyme can reduce the apparent molecular weight of SAM-6/R glycoprotein due to removal of one or more sugar residues comprising the carbohydrate moiety. In one embodiment, contact of SAM-6/R glycoprotein with a glycosidase enzyme reduces the apparent molecular weight by about 1-10 kilodaltons (kDa), for example, from about 82 kDa to 72 kDa. In another embodiment, contact of SAM-6/R glycoprotein with an O-glycosidase reduces the apparent molecular weight of SAM-6/R glycoprotein. Of course, the skilled artisan will recognize that the amount and type, if any, of carbohydrate that can be removed from SAM-6/R glycoprotein will depend upon selection of a particular glycosidase enzyme, which will in turn affect the reduction, if any, in the apparent molecular weight of SAM-6/R glycoprotein.

Glycosidases capable of removing one or more sugars of a carbohydrate moiety, or the entire carbohydrate structure, include O-glycosidases, which typically cleave sugars that comprise carbohydrate moieties linked to the oxygen (O) of serine or threonine residues, and N-glycosidases, which typically cleave sugars that comprise carbohydrate moieties linked to the nitrogen (N) of asparagine residues. Particular examples of such glycosidases are O-glycosidase, N-glycosidase F, endoglycosidase H (endo H), neuraminidase and fucosidases. O-glycosidase cleaves serine- or threonine-linked oligosaccharide. N-glycosidase F cleaves asparagine bound N-glycans when the oligosaccharide has a minimum length of the chitobiose core unit. Endo H is a glycosidase that cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from N-linked glycoproteins. Neuraminidase removes terminal acylneuraminic residues. Fucosidases remove fucose, for example, from lactose and complex carbohydrates. Such glycosidases typically have at least some specificity in terms of the sugar linkages cleaved and whether the carbohydrate moieties are O- or N-linked and can therefore be used to characterize the composition and structure of the SAM-6/R glycoprotein carbohydrate moiety (ies).

A panel of carbohydrates was screened for binding of SAM-6 antibody. In particular, (GlcNAc)2, Man3, sTn, GM4, Lac-di-Nac, β-D-galactose-3-sulfate, H type 3, Neu5Ac6Gal, GlcNacβ3Gal, α-N-acetylneuraminic acid, 3′-SL, Atri, Tk, 3′SLN, sLe^a, Btri, GA1 Pk, Gb3, sLe^x, Adi, A type 2, Tαβ, 6′-SL, Bdi, B type 2, Galβ3Gal, Tββ, H type 2, Gala1-3'Lac, Le^a 3′-O-su-Le^a, 6'-O-su-LacNAc, GlcNAcβ1-2'TF, Le^b, 3′-O-su-Le^x, Hdi, Galα4GlcNAc, Le^d (H type 1), 3′-su-LacNAc, 3′-O-su-TF, β-N-acetylneuraminic acid, Le^C, 3′-su-Le^c, GlcNAcβ1-3'LacNAc, maltose, Le^x, melibiose, di-GalNAcβ, α-D-glucose, Le^y, Galα1-3'LacNAc, 3′-SiaTF, β-D-glucose, Lac, GlcNAcα1-3'TF, core 3, α-D-galactose, LacNAc, $(Sia)_2$, core 6, β-D-galactose, TF, $(Sia)_3$, core 4, α-D-mannose, Fucα3GlcNAc, GlcNAcβ1-3'TF, 3,6-SiaTF, α-D-mannose-6-phosphate, Fucα4GlcNAc Le, Gal2βGal, 6-SiaTF, α-L-fucose, Fs-2, 6-O-su-LacNAc, YDS, β-N-acetyl-D-glucosamine, core 5, core 2, 9-OS, α-N-acetyl-D-glalactosamine (Tn), Tαα, H type 4, 7-OS, β-N-acetyl-D-glalactosamine, 3′-$SiaLe^c$, LNT, 3,6-SiaTn, β-N-acetyl-D-glucosamine-6-sulfate, Galα2Gal, LNnT and 3-SiaTn were all screened for binding to SAM-6 antibody. SAM-6 antibody did not detectably bind to any of these carbohydrates. Consequently, a SAM-6 antibody of the invention includes antibodies that bind to SAM-6/R glycoprotein but not to one or more of the aforementioned carbohydrates.

In embodiments in which antibody denoted SAM-6 specifically binds to SAM-6/R glycoprotein, the SAM-6 antibody may bind ro a region of the SAM-6/R glycoprotein that includes a carbohydrate moiety (*e.g.*, an N- or O-linked carbohydrate moiety). Treatment of

SAM-6/R glycoprotein with an O-glycosidase enzyme reduced binding of SAM-6 antibody to the glycoprotein, presumably due to removal of one, several or all sugar(s) near a SAM-6 antibody binding epitope (Figure 16). Treatment of SAM-6/R glycoprotein with an N-glycosidase F enzyme did not destroy binding of SAM-6 antibody to the glycoprotein (Figure 16). Accordingly, SAM-6 binding to SAM-6/R may be near one or more sugars comprising an O-linked carbohydrate moiety.

SAM-6 antibodies bind to deglycosylated Grp78, as disclosed in Example 12. Thus, an an O-linked carbohydrate moiety does not appear to be required for binding of SAM-6 antibodies to SAM-6 Target/Grp78. SAM-6 antibodies therefore include those that bind to deglycosylated or glycosylated Grp78.

SAM-6 also detectably bind to apoB100. Furthermore, SAM-6 detectably binds to LDL, VLDL, and deglycosylated LDL. Thus, each of these SAM-6 targets likely share a common structural epitope to which SAM-6 recognizes/binds. Accordingly, the invention provides antibodies and subsequences thereof that bind to one or more of Grp78, deglycosylated Grp78, apoB100, LDL (e.g., oxLDL), VLDL, including glycosylated or deglycosylated forms thereof.

The term "SAM-6" or "SAM-6 antibody" generally refers to antibodies and subsequences thereof with any light chain or a heavy chain variable region sequence disclosed herein. "SAM-6 antibody" specifically binds to one or more of SAM-6/R glycoprotein, Grp78, deglycosylated Grp78, apoB100, LDL (e.g., oxLDL), VLDL, or deglycosylated LDL. The term SAM-6 antibody may exclude other antibodies, for example, antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

Due to reduced SAM-6 binding to SAM-6/R glycoprotein observed after treatment of SAM-6/R glycoprotein with an O-glycosidase, SAM-6 may bind to a region of SAM-6/R glycoprotein that includes or is near to an O-linked carbohydrate moiety. SAM-6 does not appear to bind to a region of SAM-6/R glycoprotein that includes an N-linked carbohydrate moiety, as indicated by maintained SAM-6 binding to SAM-6/R glycoprotein after treatment of SAM-6/R glycoprotein with N-glycosidase F.

Representative amino acid and nucleic acid sequences of SAM-6 light and heavy chain variable regions, with the complementary determining regions (CDR1-CDR3) indicated, are as follows.

Amino acid sequence of the variable region of the light chain (V_L) of antibody SAM-6 (SEQ ID NO:13):

Ser	Tyr	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Val	Ser	Pro	Gly	Gln	Thr	Ala	Ser
1				5					10					15				
Ile	Thr	Cys	Ser	Gly	Asp	Lys	Leu	Gly	Asp	Lys	Tyr	Ala	Cys	Trp	Tyr	Gln	Gln	Lys
20					25					30					35			
Pro	Gly	Gln	Ser	Pro	Val	Leu	Val	Ile	Tyr	Gln	Asp	Ser	Lys	Arg	Pro	Ser	Gly	Ile
	40					45					50					55		
Pro	Glu	Arg	Phe	Ser	Gly	Ser	Asn	Ser	Gly	Asn	Thr	Ala	Thr	Leu	Thr	Ile	Ser	Gly
		60					65					70					75	
Thr	Gln	Ala	Met	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ala	Trp	Asp	Ser	Ser	Ile	Val
			80					85					90					95
Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Gly	Gln						

Nucleotide sequence of the variable region of the light chain (V_L) of antibody SAM-6 (SEQ ID NO:14):

tcc	tat	gtg	ctg	act	cag	cca	ccc	tca	gtg	tcc	gtg	tcc	cca	gga		45
Ser	Tyr	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Val	Ser	Pro	Gly		
1				5					10					15		

CDR 1

cag	aca	gcc	agc	atc	acc	tgc	tct	gga	gat	aaa	ttg	ggg	gat	aaa		90
Gln	Thr	Ala	Ser	Ile	Thr	Cys	Ser	Gly	Asp	Lys	Leu	Gly	Asp	Lys		
				20					25					30		

tat	gct	tgc	tgg	tat	cag	cag	aag	cca	ggc	cag	tcc	cct	gtg	ctg		135
Tyr	Ala	Cys	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Val	Leu		
				35					40					45		

CDR2

gtc	atc	tat	caa	gat	agc	aag	cgg	ccc	tca	ggg	atc	cct	gag	cga		180
Val	Ile	Tyr	Gln	Asp	Ser	Lys	Arg	Pro	Ser	Gly	Ile	Pro	Glu	Arg		
				50					55					60		

ttc	tct	ggc	tcc	aac	tct	ggg	aac	aca	gcc	act	ctg	acc	atc	agc		225
Phe	Ser	Gly	Ser	Asn	Ser	Gly	Asn	Thr	Ala	Thr	Leu	Thr	Ile	Ser		
				65					70					75		

ggg	acc	cag	gct	atg	gat	gag	gct	gac	tat	tac	tgt	cag	gcg	tgg	270
Gly	Thr	Gln	Ala	Met	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ala	Trp	
				80					85					90	

CDR3

gac	agc	agc	att	gtg	gta	ttc	ggc	gga	ggg	acc	aag	ctg	acc	gtc	cta	ggt2cag88
Asp	Ser	Ser	Ile	Val	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Gly Gln
				95												

Amino Acid sequence of the variable region of the heavy chain (V_H) of antibody SAM-6 (SEQ ID NO:15):

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg	Ser	Leu
1				5					10					15			
Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	Ala	Met	His	Trp
	20					25					30					35	
Val	Arg	Glu	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	Ala	Val	Ile	Ser	Tyr	Asp
			40					45					50				
Gly	Ser	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg
55					60					65					70		
Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp
		75					80					85					90
Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Asp	Arg	Leu	Ala	Val	Ala	Gly	Lys	Thr	Phe
				95					100					105			
Asp	Tyr																
	110																

Nucleotide sequence of the variable region of the heavy chain (V_H) of antibody SAM-6 (SEQ ID NO:16):

cag	gtg	cag	ctg	gtg	gag	tct	ggg	gga	ggc	gtg	gtc	cag	cct	ggg	45
Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	
1				5					10					15	
agg	tcc	ctg	aga	ctc	tcc	tgt	gca	gcc	tct	gga	ttc	acc	ttc	agt	90
Arg	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	
				20					25					30	

CDR1

agc	tat	gct	atg	cac	tgg	gtc	cgc	cag	gct	cca	ggc	aag	ggg	ctg	135
Ser	Tyr	Ala	Met	His	Trp	Val	Arg	Glu	Ala	Pro	Gly	Lys	Gly	Leu	

				35					40					45	
								CDR2							
gag	tgg	gtg	gca	gtt	ata	tca	tat	gat	gga	agc	aat	aaa	tac	tac	180
Glu	Trp	Val	Ala	Val	Ile	Ser	Tyr	Asp	Gly	Ser	Asn	Lys	Tyr	Tyr	
				50					55					60	
gca	gac	tcc	gtg	aag	ggc	cga	ttc	acc	atc	tcc	aga	gac	aat	tcc	225
Ala	Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	
				65					70					75	
aag	aac	acg	ctg	tat	ctg	caa	atg	aac	agc	ctg	aga	gct	gag	gac	270
Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	
				80					85					90	
										CDR3					
acg	gct	gtg	tat	tac	tgt	gcg	aga	gat	cgg	tta	gca	gtg	gct	ggt	315
Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Asp	Arg	Leu	Ala	Val	Ala	Gly	
				95					100					105	
aaa	act	ttt	gac	tac											
Lys	Thr	Phe	Asp	Tyr											
				110											

In certain embodiments, SAM-6/R glycoprotein is characterized as being expressed on a hyperproliferative cell, such as a neoplastic, cancer or tumor cell. Non-limiting examples of neoplastic, cancer and tumor cells in which SAM-6/R glycoprotein has been detected include, for example, esophagus, stomach, breast, lung, colon, pancreas, prostate, uterus and ovary. In more particular aspects, SAM-6/R glycoprotein is characterized as being expressed on tumor cell lines denoted as BXPC-3 (ATCC Deposit No. CRL-1687; P.O. Box 1549 Manassas, VA, 20108, USA) and A549 (DSMZ Deposit No. CCL185; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures), Inhoffenstrase 7 B 38124 Braunschweig, Germany).

In accordance with the invention, there are provided isolated or purified glycoprotein denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, that optionally have at least 60%, 70%, 80%, 90%, 95% or more identity with SEQ ID NO:1, or any numerical value or range within or encompassing such percent values. In one embodiment, SAM-6/R glycoprotein has a polypeptide sequence identical to all or a part of Grp78 sequence as set forth in SEQ ID NO:1. In particular aspects, SAM-6/R glycoprotein has a

polypeptide sequence identical to all or a part of one or more of the following amino acid sequences (SEQ ID NOs:2-12): NGRVEIIANDQGNRITPSYVAFTPEGER; NQLTSNPENTVFDAKR; TWNDPSVQQDIKFLPFKVVEKKTKPYIQVDIGGGQTKTFAPEEISAMVLTK; KVTHAVVTVPAYFNDAQRQATKDAGTIAGLNVMR; VMEHFIK; AKFEELNMDLFRSTMKPVQKVLEDSDLK; EFFNGKEPSR; VYEGERPLTKDNHLLGTFDLTGIPPAPR; LTPEEIER; IDTRNELESYAYSLK; or LYGSAGPPPTGEEDTAEKDEL. In another embodiment, SAM-6/R glycoprotein has a carbohydrate moiety linked thereto that is distinct from a Grp78 carbohydrate moiety. In particular aspects, the carbohydrate moiety is an N-linked or O-linked moiety.

The terms "identical" or "identity," mean that two or more referenced entities are the same. Thus, where two protein sequences are identical, they have the same amino acid sequence, at least within the referenced region or portion. Where two nucleic acid sequences are identical, they have the same polynucleotide sequence, at least within the referenced region or portion. An "area of identity" refers to a portion of two or more referenced entities that are the same. Thus, where two protein or nucleic acid sequences are identical over one or more sequence regions they share identity within that region.

The terms "homologous" or "homology" mean that two or more referenced entities share at least partial identity over a given region or portion. "Substantial homology" means that a molecule is structurally or functionally conserved such that it has or is predicted to have at least partial structure or function of one or more of the structures or functions (*e.g.*, a biological function) of the reference molecule, or relevant/corresponding region or portion of the reference molecule to which it shares homology. Exemplary homology are polypeptides having amino acid sequences with 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or more sequence identity to a reference polypeptide. A polypeptide with substantial homology has or is predicted to have at least partial activity as the reference polypeptide. For example, in a particular embodiment, a SAM-6/R glycoprotein having one or more modifications (*e.g.*, substitutions, deletions or additions of carbohydrate moiety or amino acid) that retains at least partial binding to SAM-6 is considered to have substantial homology to SAM-6/R glycoprotein.

The terms "protein," "polypeptide" and "peptide" are used interchangeably herein to refer to two or more amino acids, or "residues," covalently linked by an amide bond or equivalent. Polypeptides can be various lengths and the amino acids may be linked by non-natural and non-amide chemical bonds including, for example, those formed with glutaraldehyde, N-

hydroxysuccinimide esters, bifunctional maleimides, or N, N'-dicyclohexylcarbodiimide (DCC). Non-amide bonds include, for example, ketomethylene, aminomethylene, olefin, ether, thioether and the like (see, *e.g.,* Spatola in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357 (1983), "Peptide and Backbone Modifications," Marcel Decker, NY).

The term "isolated" used as a modifier of a composition means that the composition is made by the hand of man or is separated from one or more other components in their naturally occurring *in vivo* environment. Generally, compositions so separated are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein, nucleic acid, lipid, carbohydrate, cell membrane. Thus, an isolated composition is substantially separated from other biological components in the cell of the organism in which the composition naturally occurs, or from the artificial medium in which it is produced (*e.g.,* synthetically or through cell culture). For example, an isolated polypeptide is substantially separated from other polypeptides and nucleic acid and does not include a library of polypeptides or polynucleotides present among millions of polypeptide or nucleic acid sequences, such as a polypeptide, genomic or cDNA library, for example. An isolated nucleic acid is substantially separated from other polypeptides and nucleic acid and does not include a library of polypeptides or polynucleotides present among millions of polypeptide or nucleic acid sequences, such as a polypeptide, genomic or cDNA library, for example. The term "isolated" does not exclude alternative physical forms of the composition, for example, an isolated protein could include protein multimers, post-translational modifications (*e.g.,* glycosylation, phosphorylation) or derivatized forms.

The term "purified" used as a modifier of a composition refers to a composition free of most or all of the materials with which it typically associates with in nature. Thus, a protein separated from cells is considered to be substantially purified when separated from cellular components by standard methods while a chemically synthesized nucleic acid sequence is considered to be substantially purified when separated from its chemical precursors. Purified therefore does not require absolute purity. Furthermore, a "purified" composition can be combined with one or more other molecules. Thus, the term "purified" does not exclude combinations of compositions.

"Purified" proteins and nucleic acid include proteins and nucleic acids produced by standard purification methods. The term also includes proteins and nucleic acids produced by recombinant expression in a host cell as well as chemical synthesis. "Purified" can also refer to a

composition in which the level of contaminants is below a level that is acceptable to a regulatory agency for administration to a human or non-human animal, for example, the Food and Drug administration (FDA).

Substantial purity can be at least about 60% or more of the molecule by mass. Purity can also be about 70% or 80% or more, and can be greater, for example, 90% or more. Purity can be less, for example, in a pharmaceutical carrier the amount of a molecule by weight % can be less than 60% but the relative proportion of the molecule compared to other components with which it is normally associated with will be greater. Purity can be determined by any appropriate method, including, for example, UV spectroscopy, chromatography (*e.g.*, HPLC, gas phase), gel electrophoresis (*e.g.*, silver or coomassie staining) and sequence analysis (peptide and nucleic acid).

SAM-6/R glycoprotein is expressed on malignant and non-malignant, neoplastic, tumor and cancer cells. For example, SAM-6/R glycoprotein is expressed on malignant and non-malignant gastric tissue, lung squamous cell carcinoma, lung adenocarcinoma, melanomas and nasal cancer cells.

SAM-6/R glycoprotein is also secreted by tumor, cancer or neoplastic cells. For example, SAM-6/R glycoprotein is secreted by A549 cells.

SAM-6/R glycoprotein is also expressed on tumors at various stages and grades. For example, SAM-6/R glycoprotein was detected on all of stages IA, IB, IIA, IIB, IIIA, IIIB and IV, and grades G1, G2 and G3, of lung squamous cell carcinoma and adenocarcinoma.

SAM-6/R glycoprotein is additionally expressed on tumor metastasis. For example, SAM-6/R glycoprotein was detected on lung squamous cell carcinoma and adenocarcinoma metastasis to lymph node and brain; SAM-6/R glycoprotein was detected on breast cancer (invasive ductal) metastasis to lymph node; SAM-6/R glycoprotein was detected on colon adenocarcinoma metastasis to liver and lymph node; SAM-6/R glycoprotein was detected on stomach adenocarcinoma (intestinal and diffuse) metastasis to lymph node; SAM-6/R glycoprotein was detected on pancreas adenocarcinmoa metastasis to lymph node; SAM-6/R glycoprotein was detected on head and neck squamous cell carcinoma metastasis to lymph node; and SAM-6/R glycoprotein was detected on melanoma metastasis to rectum, esophagus, skin, parotid gland, colon, adrenal gland and nasal epithelium.

SAM-6/R glycoprotein is further expressed on tumor cell lines. For example, SAM-6/R glycoprotein is expressed on tumor cell line denoted as BXPC-3 (ATCC Deposit No. CRL-1687; P.O. Box 1549 Manassas, VA, 20108, USA) A549 (DSMZ Deposit No. CCL185; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures), Inhoffenstrase 7 B 38124 Braunschweig, Germany) melanoma cell lines CRL1424 and HTB-69, and nasal cancer cells (RPMI2650). Isolated or purified SAM-6/R glycoprotein can be obtained from these tumors, metastasis, cell lines and other cells (primary isolates or passaged or immortalized cell lines) using the purification methods disclosed herein or known in the art.

In accordance with the invention, there are provided antibodies that specifically bind to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, immunogenic subsequences thereof, as well as cells that express the foregoing. Such antibodies include antibodies that specifically bind to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, antibodies whose affinity is reduced when SAM-6/R glycoprotein is treated with a glycosidase, and antibodies that specifically bind to Grp78 and apoB100.

SAM-6/R glycoprotein is typically not expressed on non-cancer cells. For example, SAM-6/R glycoprotein was not detected on fresh frozen (FDA standard) normal human tissue from adrenal gland, cerebellum, cerebrum, pituitary, breast, colon, esophagus, heart, kidney, liver, lung, skeletal muscle, mesothileal, peripheral nerve, sciatic nerve, trigeminal nervew, ovary, pancreas, placenta, prostate, salivary gland, skin, small intestine, spleen, stomach, testis, thymus, thyroid, tonsil, uterus, cervix, or bone marrow. SAM-6/R glycoprotein was also not detected on lymphocytes, granulocytes, human fibroblasts and normal nasal cells (HNEPC). Thus, antibodies that specifically bind to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, are not expected to bind one or more of the foregoing normal human tissue types, cells derived or that are considered representative of such normal human tissue types, nor lymphocytes, granulocytes, human fibroblasts or normal nasal cells (HNEPC).

Antibodies that specifically bind to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or

deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, include isolated antibodies, purified antibodies and antibody subsequences. In one embodiment, an antibody that binds to the SAM-6/R glycoprotein extracellular domain is provided. In another embodiment, an antibody whose binding is reduced when a sugar of an N- or O-linked carbohydrate moiety of SAM-6/R glycoprotein is removed is provided. In a further embodiment, an antibody that binds to SAM-6/R glycoprotein that is distinct from Grp78 is provided. In an additional embodiment, an antibody is provided that specifically binds to SAM-6/R glycoprotein, but exhibits reduced binding after SAM-6/R glycoprotein is contacted with a glycosidase. In one aspect, O-glycosidase treatment of SAM-6/R glycoprotein reduces or destroys binding of the antibody to SAM-6/R. In another aspect, N-glycosidase F treatment of SAM-6/R glycoprotein does not reduce or destroy binding of the antibody to SAM-6/R. In yet another embodiment, an antibody binds to one or more of glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL. In a still further embodiment, an antibody is provided that does not bind to an epitope comprising an N- or O-linked carbohydrate moiety of SAM-6/R glycoprotein.

Invention antibodies that specifically bind to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, include thos distinct from SAM-6 antibody (e.g., as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18)). Invention antibodies also include antibodies that do not have a heavy or a light chain variable sequence identical to heavy or light chain variable sequences of SAM-6 antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

Antibody heavy chain variable region (V_H) alone can bind to a target antigen. In particular embodiments, a heavy chain variable region (V_H) alone can bind to one or more of a glycosylated or deglycosylated SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, a glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL, glycosylated or deglycosylated VLDL or glycosylated or deglycosylated oxidized LDL.

In a particular embodiment, a SAM-6 antibody as defined herein has a heavy chain sequence with 100% identity to a heavy chain variable region sequence or the third CDR (CDR3;

ARDRLAVAGRPFDY; SEQ ID NO:17) within a heavy chain variable region amino acid sequence (SEQ ID NO: 18) set forth as:

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<------------------------------------------  FR1 - IMGT
                                  G   G       G   V   V   Q   P
... ... ... ... ... ... ... ... ggg gga ... ggc gtg gtc cag cct

------------------------------------------>  _______________
 G   R   S   L   R   L   S   C   A   A   S   G   F   T   F
ggg agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttc

__  CDR1 - IMGT  _______________ <--------------------------
 S   S   Y   A                   M   H   W   V   R   Q   A
agt agc tat gct ... ... ... ... atg cac tgg gtc cgc cag gct

FR2 - IMGT  ------------------------>  _______________  CDR2
 P   G   K   G   L   E   W   V   A   V   I   S   Y   D   G
cca ggc aag ggg ctg gag tgg gtg gca gtt ata tca tat gat gga

- IMGT  __________ <-------------------------------------
 S   N   K           Y   Y   A   D   S   V   K       G   R
agc aat aaa ... ... tac tac gca gac tcc gtg aag ... ggc cga

------------------------------  FR3 - IMGT  ---------------
 F   T   I   S   R   D   N   S   K   N   T   L   Y   L   Q
ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg caa

-------------------------------------------------------->  ____
 M   N   S   L   R   A   E   D   T   A   V   Y   Y   C   A
atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt gcg

_________________  CDR3 - IMGT  _________________
 R   D   R   L   A   V   A   G   R   P   F   D   Y   W   G
aga gat cgg tta gca gtg gct ggt aga cct ttt gac tac tgg ggc


 Q   G   T   L   V   T   V   S   S   G
cag gga acc ctg gtc acc gtc tcc tca ggg
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Antibodies of the invention include antibodies having one or more activities or functions of SAM-6 antibody. In one embodiment, an antibody specifically binds to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, LDL (*e.g.,* oxLDL), glycosylated or deglycosylated VLDL. In another embodiment, an antibody exhibits greater binding to a neoplastic, tumor or cancer, or metastasis cell, than a corresponding non-neoplastic, non-tumor or non-cancer or non-metastasis cell. In a further embodiment, binding of a SAM-6/R glycoprotein antibody to a cell expressing SAM-6/R glycoprotein inhibits, decreases or reduces cell growth or proliferation, or stimulates or induces death, lysis or apoptosis of the cell.

Non-limiting cells that express SAM-6/R glycoprotein to which the antibodies bind include malignant and non-malignant gastric tissue cell, lung squamous cell carcinoma, lung adenocarcinoma cell, of any stage (e.g., stages IA, IB, IIA, IIB, IIIA, IIIB or IV) or grade (e.g.,

grades G1, G2 or G3), melanomas and nasal cancer cells. Additional non-limiting cells that express SAM-6/R glycoprotein to which the antibodies bind include tumor metastasis, such as lung squamous cell carcinoma and adenocarcinmoa metastasis to lymph node and brain; breast cancer (invasive ductal) metastasis to lymph node; colon adenocarcinoma metastasis to liver and lymph node; SAM-6/R glycoprotein was detected on stomach adenocarcinoma (intestinal and diffuse) metastasis to lymph node; pancreas adenocarcinmoa metastasis to lymph node; head and neck squamous cell carcinoma metastasis to lymph node; and melanoma metastasis to rectum, esophagus, skin, parotid gland, colon, adrenal gland and nasal epithelium.

Further non-limiting cells that express SAM-6/R glycoprotein to which the antibodies bind include tumor cell lines, such as BXPC-3 (ATCC Deposit No. CRL-1687; P.O. Box 1549 Manassas, VA, 20108, USA), A549 (DSMZ Deposit No. CCL185; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures), Inhoffenstrase 7 B 38124 Braunschweig, Germany), melanoma cells CRL1424 and HTB-69, and nasal cancer cells RPMI2650.

Thus, in additional embodiments, binding of SAM-6/R glycoprotein antibody to a neoplastic, tumor or cancer, or metastasis cell expressing SAM-6/R glycoprotein inhibits, decreases or reduces cell growth or proliferation, or stimulates or induces cell death, lysis or apoptosis. In still another embodiment, binding of SAM-6/R glycoprotein antibody to BXPC-3, A549, CRL1424, HTB-69, or RPMI2650 cells inhibits, decreases or reduces BXPC-3, A549, CRL1424, HTB-69, or RPMI265 cell growth or proliferation, or stimulates or induces BXPC-3, A549, CRL1424, HTB-69, or RPMI265 cell death, lysis or apoptosis. In particular aspects, cell growth or proliferation is inhibited, decreased or reduced at least 20%, 30%, 40%, 50%, 60%, 75%, or more relative to a control (untreated) cell, or any numerical value or range within or encompassing such percent values. In further particular aspects, cell death, lysis or apoptosis is at least 20%, 30%, 40%, 50%, 60%, 75%, or more relative to a control (untreated) cell, or any numerical value or range within or encompassing such percent values. In yet a further embodiment, binding of the antibody to cells expressing the glycoprotein causes activation of a caspase (*e.g.*, caspase-3, caspase-7, caspase-8 or caspase-9).

Antibodies of the invention include polyclonal and monoclonal antibodies. The term "monoclonal," when used in reference to an antibody refers to an antibody that is based upon, obtained from or derived from a single clone, including any eukaryotic, prokaryotic, or phage clone. A "monoclonal" antibody is therefore defined herein structurally, and not the method by which it is produced.

Antibodies of the invention can belong to any antibody class, IgM, IgG, IgE, IgA, IgD, or subclass. Exemplary subclasses for IgG are IgG_1, IgG_2, IgG_3 and IgG_4.

Antibodies of the invention can have kappa or lambda light chain sequences, either full length as in naturally occurring antibodies, mixtures thereof (i.e., fusions of kappa and lambda chain sequences), and subsequences/fragments thereof. Naturally occurring antibody molecules contain two kappa or two lambda light chains. The primary difference between kappa and lambda light chains is in the sequences of the constant region.

In accordance with the invention, there are provided antibodies that specifically bind to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, and that partially or fully compete for binding of SAM-6 to glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof. In one embodiment, an antibody that inhibits binding of one or more of SAM-6 to glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, is provided. In various aspects, the antibody competitively inhibits binding by at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the binding of SAM-6 to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, or any numerical value or range within or encompassing such percent values. In another embodiment, an antibody that prevents or blocks binding of SAM-6 to oen or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, is provided.

Invention antibodies that compete for binding of the SAM-6 antibody to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, can have the binding specificity of SAM-6 antibody. Thus, a SAM-6/R glycoprotein antibody that competes with SAM-6 antibody for binding to one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated

VLDL, or a subsequence thereof, can specifically bind to an epitope (e.g., a structure) of one or more of glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof. A SAM-6/R glycoprotein antibody may also be able to compete with SAM-6 antibody for binding to glycosylated or deglycosylated LDL or oxLDL. Accordingly, antibodies are provided that specifically bind to glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, and that partially or fully compete for binding of SAM-6 antibody to glycosylated or deglycosylated SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, including antibodies that bind to the same epitope, a part of the same epitope or a sequence or structure of SAM-6/R glycoprotein, glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL (*e.g.*, oxLDL), glycosylated or deglycosylated VLDL, or a subsequence thereof, as SAM-6 antibody.

Antibodies that compete for binding of the SAM-6 antibody to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, or glycosylated or deglycosylated forms, or a subsequence thereof, can be screened and identified using a conventional competition binding assays. Screened antibodies are selected based upon an ability to compete for binding of the SAM-6 antibody to SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, or glycosylated or deglycosylated forms, or a subsequence thereof. The ability of an antibody to compete for, or inhibit, prevent or block binding of SAM-6 to SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, can be determined by various assays know in the art, including enzyme linked immunosorbent assay (ELISA), immunoprecipitation, wester blot, etc.

Invention antibodies include antibodies having binding affinity of SAM-6 antibody. The binding affinity of such antibodies may differ from SAM-6 antibody (i.e., have greater or less affinity for one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms, or a subsequence thereof) and therefore, the antibodies can vary in their ability to compete for binding to one or more of SAM-6 glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof. Invention antibodies thus include antibodies that have greater or less affinity for one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, than SAM-6 antibody. For example, SAM-6/R

glycoprotein antibody of the invention may have an affinity greater or less than 2-5, 5-10, 10-100, 100-1000 or 1000-10,000-fold affinity, or any numerical value or range within or encompassing such values, as the reference antibody (*e.g.*, SAM-6 antibody). Specific non-limiting SAM-6/R glycoprotein antibodies have a binding affinity for SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, within about 1-5,000 fold of the binding affinity of SAM-6. Additional specific non-limiting antibodies have a binding affinity for SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, is within about K_d 10^{-2} M to about K_d 10^{-15} M, or within about K_d 10^{-5} M to about K_d 10^{-12} M, of SAM-6 for SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, or any numerical value or range within or encompassing such values. In more particular embodiments, a binding affinity for SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, with a dissociation constant (KD) less than $5x10^{-2}$ M, 10^{-2} M, $5x10^{-3}$ M, 10^{-3} M $5x10^{-4}$ M, 10^{-4} M $5x10^{-5}$ M, 10^{-5} M $5x10^{-6}$ M, 10^{-6} M $5x10^{-7}$ M, 10^{-7} M $5x10^{-8}$ M, 10^{-8} M $5x10^{-9}$ M, 10^{-9} M $5x10^{-10}$ M, 10^{-10} M $5x10^{-11}$ M, 10^{-11} M $5x10^{-12}$ M, 10^{-12} M $5x10^{-13}$ M, 10^{-13} M $5x10^{-14}$ M, 10^{-14} M $5x10^{-15}$ M, and 10^{-15} M, or any numerical value or range within or encompassing such values.

Binding affinity can be determined by association (K_a) and dissociation (K_d) rate. Equilibrium affinity constant, KD, is the ratio of K_a/K_d. Association (K_a) and dissociation (K_d) rates can be measured using surface plasmon resonance (SPR) (Rich and Myszka, *Curr. Opin. Biotechnol.* 11:54 (2000); Englebienne, *Analyst.* 123:1599 (1998)). Instrumentation and methods for real time detection and monitoring of binding rates are known and are commercially available (BiaCore 2000, Biacore AB, Upsala, Sweden; and Malmqvist, *Biochem. Soc. Trans.* 27:335 (1999)). KD values can be defined as the antibody concentration required to saturate one half (50%) of the binding sites on SAM-6/R glycoprotein.

Methods of producing polyclonal and monoclonal antibodies are known in the art. For example, SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, or an immunogenic fragment thereof, optionally conjugated to a carrier such as keyhole limpet hemocyanin (KLH) or ovalbumin (*e.g.*, BSA), or mixed with an adjuvant such as Freund's complete or incomplete adjuvant, and used to immunize an animal. Using conventional hybridoma technology, splenocytes from immunized animals that respond to SAM-6/R glycoprotein can be isolated and fused with myeloma cells. Monoclonal antibodies produced by the hybridomas can be screened for reactivity with SAM-6/R glycoprotein or an immunogenic fragment thereof.

Animals that may be immunized include mice, rats, rabbits, goats, sheep, cows or steer, guinea pigs or primates. Initial and any optional subsequent immunization may be through intravenous, intraperitoneal, intramuscular, or subcutaneous routes. Subsequent immunizations may be at the same or at different concentrations of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, preparation, and may be at regular or irregular intervals.

Animals include those genetically modified to include human IgG gene loci, which can therefore be used to produce human antibodies. Transgenic animals with one or more human immunoglobulin genes that do not express endogenous immunoglobulins are described, for example in, U.S. Patent No. 5,939,598. Additional methods for producing human polyclonal antibodies and human monoclonal antibodies are described (see, *e.g.*, Kuroiwa *et al.*, *Nat. Biotechnol.* 20:889 (2002); WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598). An overview of the technology for producing human antibodies is described in Lonberg and Huszar (*Int. Rev. Immunol.* 13:65 (1995)).

Antibodies can also be generated using other techniques including hybridoma, recombinant, and phage display technologies, or a combination thereof (see U.S. Patent Nos. 4,902,614, 4,543,439, and 4,411,993; see, also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Harlow *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2nd ed. 1988).

Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; W091/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunol.* 28:489 (1991); Studnicka *et al.*, *Protein Engineering* 7:805 (1994); Roguska. *et al.*, *Proc. Nat'l. Acad. Sci. USA* 91:969 (1994)), and chain shuffling (U.S. Patent No. 5,565,332). Human consensus sequences (Padlan, *Mol. Immunol.* 31:169 (1994); and Padlan, *Mol. Immunol.* 28:489 (1991)) have previously used to produce humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); and Presta *et al.*, *J. Immunol.* 151:2623 (1993)).

Methods for producing chimeric antibodies are known in the art (*e.g.*, Morrison, *Science* 229:1202 (1985); Oi *et al.*, *BioTechniques* 4:214 (1986); Gillies *et al.*, *J. Immunol. Methods* 125:191 (1989); and U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397). Chimeric antibodies in which a variable domain from an antibody of one species is substituted for the variable domain

of another species are described, for example, in Munro, *Nature* 312:597 (1984); Neuberger *et al.*, *Nature* 312:604 (1984); Sharon *et al.*, *Nature* 309:364 (1984); Morrison *et al.*, *Proc. Nat'l. Acad. Sci. USA* 81:6851 (1984); Boulianne *et al.*, *Nature* 312:643 (1984); Capon *et al.*, *Nature* 337:525 (1989); and Traunecker *et al.*, *Nature* 339:68 (1989).

Suitable techniques that additionally may be employed in antibody methods include affinity purification, non-denaturing gel purification, HPLC or RP-HPLC, size exclusion, purification on protein A column, or any combination of these techniques. The antibody isotype can be determined using an ELISA assay, for example, a human Ig can be identified using mouse Ig-absorbed anti-human Ig.

SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, suitable for generating antibodies can be produced by any of a variety of standard protein purification or recombinant expression techniques known in the art. For example, SAM-6/R glycoprotein can be obtained from cells, such as BXPC-3 cells (ATCC Deposit No. CRL-1687; P.O. Box 1549 Manassas, VA, 20108, USA) or A549 (DSMZ Deposit No. CCL185; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures), Inhoffenstrase 7 B 38124 Braunschweig, Germany).

Forms of protein suitable for generating an immune response include peptide subsequences of full length protein, such as an immunogenic fragment. Additional forms of protein include preparations or cell extracts or fractions, partially purified SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, as well as whole cells that express SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, or preparations of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, expressing cells.

In accordance with the invention, further provided are methods of producing antibodies that specifically bind to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated. In one embodiment, a method includes administering a polypeptide having a molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, optionally having at least one O-linked carbohydrate moiety, and has at least partial sequence homology with Grp78, or a fragment thereof, to an animal, screening the animal for expression of an antibody that binds to the polypeptide, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, eglycosylated or deglycosylated,

selecting an animal that produces an antibody that binds to the polypeptide, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, and isolating the antibody from the selected animal. In another embodiment, a method includes administering a polypeptide having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, optionally having at least one N- or O-linked carbohydrate moiety, and having at least partial sequence homology with Grp78, or a fragment thereof, to an animal, screening the animal for expression of an antibody that binds to the polypeptide, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, selecting an animal that produces an antibody that binds to the polypeptide, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, and isolating the antibody from the selected animal. In a further embodiment, a method includes administering a polypeptide having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, having at least one N- or O-linked carbohydrate moiety, and having at least partial sequence homology with Grp78, or a fragment thereof to an animal capable of expressing a human immunoglobulin; isolating spleen cells from an animal that produces antibody that binds to the polypeptide or the fragment thereof, fusing the spleen cells with a myeloma cell to produce a hybridoma, and screening the hybridoma for expression of an antibody that binds to the polypeptide having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis, having at least one N- or O-linked carbohydrate moiety, and having at least partial sequence homology with Grp78, or the fragment thereof. In various aspects, the polypeptide fragment includes a portion of the polypeptide with an N- or O-linked carbohydrate moiety.

Invention methods include producing antibodies distinct from SAM-6 antibody that have one or more functions or activities of SAM-6 antibody. Exemplary functions or activities include, for example, binding to SAM-6/R glycoprotein (*e.g.*, extracellular domain), Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof; binding to an epitope comprising a SAM-6/R, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL, or an immunogenic fragment thereof; competing for binding of SAM-6 antibody to SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof; competing for binding of SAM-6 antibody to SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof; having binding affinity of SAM-6 antibody (*e.g.*, greater or less affinity for SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof), binding to a cell

expressing any of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof; binding to a cell expressing SAM-6/R glycoprotein and decreasing or reducing cell growth or proliferation, or stimulating or inducing death, lysis or apoptosis of the cell (*e.g.*, a neoplastic, tumor or cancer, or metastasis cell); binding to BXPC-3 or A549 cells and inhibiting BXPC-3 or A549 cell growth or proliferation, or stimulating or inducing BXPC-3 or A549 cell death, lysis or apoptosis; causing activation of a caspase (*e.g.*, caspase-3, caspase-7, caspase-8 or caspase-9). Exemplary functions or activities also include SAM-6/R glycoprotein binding sensitivity or insensitivity to glycosidases, for example, O-glycosidase enzyme treatment of SAM-6/R glycoprotein reducing or destroying binding of the antibody to SAM-6/R glycoprotein, and N-glycosidase F enzyme treatment of SAM-6/R glycoprotein not reducing or destroying binding of the antibody to SAM-6/R glycoprotein.

In accordance with the invention, also provided are modified forms of proteins, antibodies, nucleic acids, and other compositions, provided that the modified form retains, at least a part of, a function or activity of the unmodified or reference protein, nucleic acid, or antibody. For example, a modified SAM-6/R glycoprotein (*e.g.*, a subsequence or fragment) can be used as an immunogen to produce antibodies that specifically bind to SAM-6/R glycoprotein. A modified SAM-6/R glycoprotein antibody (*e.g.*, a subsequence or fragment) can be used in the invention treatment, diagnostic, screening and detection methods.

As used herein, the term "modify" and grammatical variations thereof, means that the composition deviates from a reference composition. Such modified proteins, nucleic acids and other compositions may have greater or less activity than or a distinct function from a reference unmodified protein, nucleic acid, or composition.

Modifications include amino acid and carbohydrate moiety substitutions, additions and deletions, which can be referred to as "variants." Specific non-limiting examples of amino acid modifications include protein subsequences and fragments. Exemplary SAM-6/R glycoprotein subsequences and fragments include a portion of the SAM-6/R glycoprotein comprising an N- or O-linked carbohydrate moiety, the carbohydrate moiety optionally distinct from a carbohydrate moiety of Grp78. Exemplary SAM-6/R glycoprotein subsequences and fragments also include an immunogenic portion of SAM-6/R glycoprotein, for example, a portion of SAM-6/R glycoprotein that includes one or more N- or O-linked carbohydrate moiety(ies). Exemplary SAM-6/R glycoprotein subsequences and fragments further include a portion of SAM-6/R glycoprotein that binds to SAM-6 antibody. Specific non-limiting examples of carbohydrate moiety modifications

include SAM-6/R glycoprotein having one or more sugar residues deleted (an O-linked moiety or a sugar thereof) that reduces or destroys binding to SAM-6 antibody or one or more sugar residues deleted (N-linked moiety or a sugar thereof) that does not reduce or destroy binding to SAM-6 antibody.

As used herein, the term "subsequence" or "fragment" means a portion of the full length molecule. A subsequence of a SAM-6/R glycoprotein has one or more less amino acids than a full length SAM-6/R glycoprotein (*e.g.* one or more internal or terminal amino acid deletions from either amino or carboxy-termini). A subsequence of an antibody has one or more less amino acids than a full length antibody. A nucleic acid subsequence has at least one less nucleotide than a full length comparison nucleic acid sequence. Subsequences therefore can be any length up to the full length native molecule.

Exemplary antibody subsequences and fragments of the invention include Fab, Fab', $F(ab')_2$, Fv, Fd, single-chain Fv (scFv), disulfide-linked Fvs (sdFv), V_L, V_H, trispecific (Fab_3), bispecific (Fab_2), diabody ($(V_L\text{-}V_H)_2$ or $(V_H\text{-}V_L)_2$), triabody (trivalent), tetrabody (tetravalent), minibody ($(scFv\text{-}C_H3)_2$), bispecific single-chain Fv (Bis-scFv), IgGdeltaCH2, scFv-Fc and $(scFv)_2$-Fc. Such subsequences and fragments can have the binding affinity as the full length antibody, the binding specificity as the full length antibody, or one or more activities or functions of as a full length antibody, *e.g.*, a function or activity of SAM-6 antibody. The terms "functional subsequence" and "functional fragment" when referring to an antibody refers to a portion of an antibody that retains at least a part of one or more functions or activities as an intact reference antibody, *e.g.*, a function or activity of SAM-6 antibody. For example, an antibody subsequence that binds to one or more of SAM-6 glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or an immunogenic fragment thereof is considered a functional subsequence.

Antibody subsequences and fragments can be combined. For example, a V_L or V_H subsequences can be joined by a linker sequence thereby forming a V_L-V_H chimera. A combination of single-chain Fvs (scFv) subsequences can be joined by a linker sequence thereby forming a scFv - scFv chimera. Antibody subsequences and fragments include single-chain antibodies or variable region(s) alone or in combination with all or a portion of other subsequences.

Antibody subsequences and fragments can be prepared by proteolytic hydrolysis of the antibody, for example, by pepsin or papain digestion of whole antibodies. Antibody subsequences and fragments produced by enzymatic cleavage with pepsin provide a 5S fragment

denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and the Fc fragment directly (see, *e.g.,* U.S. Patent Nos. 4,036,945 and 4,331,647; and Edelman *et al.*, *Methods Enymol.* 1:422 (1967)). Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic or chemical may also be used.

Proteins and antibodies, as well as subsequences and fragments thereof, can be produced by genetic methodology. Such techniques include expression of all or a part of the gene encoding the protein or antibody into a host cell such as Cos cells or *E. coli.* The recombinant host cells synthesize full length or a subsequence, for example, an scFv (see, *e.g.,* Whitlow *et al.*, In: Methods: A Companion to Methods in Enzymology 2:97 (1991), Bird *et al.*, *Science* 242:423 (1988); and U.S. Patent No. 4,946,778). Single-chain Fvs and antibodies can be produced as described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods Enzymol.* 203:46 (1991); Shu *et al.*, *Proc. Natl. Acad. Sci. USA* 90:7995 (1993); and Skerra *et al.*, *Science* 240:1038 (1988).

Modified proteins also include one or more D-amino acids substituted for L-amino acids (and mixtures thereof), structural and functional analogues, for example, peptidomimetics having synthetic or non-natural amino acids or amino acid analogues and derivatized forms. Modifications include cyclic structures such as an end-to-end amide bond between the amino and carboxy-terminus of the molecule or intra- or inter-molecular disulfide bond.

Modified proteins further include amino acid substitutions. In particular embodiments, a modified protein has one or a few conservative or non-conservative substitutions. Such proteins that include amino acid substitutions can be encoded by a nucleic acid. Consequently, nucleic acid sequences encoding proteins that include amino acid substitutions are also provided.

A "conservative substitution" is the replacement of one amino acid by a biologically, chemically or structurally similar residue. Biologically similar means that the substitution does not destroy a biological activity, *e.g.,* SAM-6, Grp78, apoB100, LDL (*e.g.,* oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL binding activity. Structurally similar means that the amino acids have side chains with similar length, such as alanine, glycine and serine, or a similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic or hydrophobic. Particular examples include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one

polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, serine for threonine, and the like.

Modified forms include derivatized sequences, for example, amino acids in which free amino groups form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups; the free carboxy groups from salts, methyl and ethyl esters; free hydroxl groups that form O-acyl or O-alkyl derivatives, as well as naturally occurring amino acid derivatives, for example, 4-hydroxyproline, for proline, 5-hydroxylysine for lysine, homoserine for serine, ornithine for lysine, etc. Modifications can be produced using methods known in the art (*e.g.*, PCR based site-directed, deletion and insertion mutagenesis, chemical modification and mutagenesis, cross-linking, etc.).

Modified forms of protein (*e.g.*, antibody), nucleic acid, and other compositions include additions and insertions. For example, an addition can be the covalent or non-covalent attachment of any type of molecule to a protein (*e.g.*, antibody), nucleic acid or other composition. Typically additions and insertions confer a distinct function or activity.

Additions and insertions include fusion (chimeric) polypeptide or nucleic acid sequences, which is a sequence having one or more molecules not normally present in a reference native (wild type) sequence covalently attached to the sequence. A particular example is an amino acid sequence of another protein (*e.g.*, antibody) to produce a multifunctional protein (*e.g.*, multispecific antibody).

In accordance with the invention, there are provided proteins, antibodies, nucleic acids, and other compositions that include a heterologous domain. Heterologous domains can be an amino acid addition or insertion, but are not restricted to amino acid residues. Thus, a heterologous domain can consist of any of a variety of different types of small or large functional moieties. Such moieties include nucleic acid, peptide, carbohydrate, lipid or small organic compounds, such as a drug (*e.g.*, a cell proliferative agent), metals (gold, silver), etc.

Particular non-limiting examples of heterologous domains include, for example, tags, detectable labels and cytotoxic agents. Specific examples of tags and detectable labels include enzymes (horseradish peroxidase, urease, catalase, alkaline phosphatase, beta-galactosidase, chloramphenicol transferase); enzyme substrates; ligands (*e.g.*, biotin); receptors (avidin); radionuclides (*e.g.*, C^{14}, S^{35}, P^{32}, P^{33}, H^{3}, I^{125}, I^{131}, gallium-67 and 68, scantium-47, indium-111, radium-223); T7-, His-, myc-, HA- and FLAG-tags; electron-dense reagents; energy transfer molecules; paramagnetic labels; fluorophores (fluorescein, rhodamine, phycoerthrin);

chromophores; chemi-luminescent (imidazole, luciferase); and bio-luminescent agents. Specific examples of cytotoxic agents include diptheria, toxin, cholera toxin and ricin.

Additional examples of heterologous domains include, for example, anti-cell proliferative agents (*e.g.,* anti-neoplastic, anti-tumor or anti-cancer, or anti-metastasis agents). Specific non-limiting examples of anti-cell proliferative agents are disclosed herein and known in the art.

Linker sequences may be inserted between the protein (*e.g.,* antibody), nucleic acid, or other composition and the addition or insertion (*e.g.,* heterologous domain) so that the two entities maintain, at least in part, a distinct function or activity. Linker sequences may have one or more properties that include a flexible structure, an inability to form an ordered secondary structure or a hydrophobic or charged character which could promote or interact with either domain. Amino acids typically found in flexible protein regions include Gly, Asn and Ser. Other near neutral amino acids, such as Thr and Ala, may also be used in the linker sequence. The length of the linker sequence may vary (see, *e.g.,* U.S. Patent No. 6,087,329). Linkers further include chemical cross-linking and conjugating agents, such as sulfo-succinimidyl derivatives (sulfo-SMCC, sulfo-SMPB), disuccinimidyl suberate (DSS), disuccinimidyl glutarate (DSG) and disuccinimidyl tartrate (DST).

Further examples of additions include glycosylation, fatty acids, lipids, acetylation, phosphorylation, amidation, formylation, ubiquitinatation, and derivatization by protecting/blocking groups and any of numerous chemical modifications. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered to be within the scope of the invention.

Such modified sequences can be made using recombinant DNA technology via cell expression or in vitro translation. Polypeptide and nucleic acid sequences can also be produced by chemical synthesis using methods known in the art, for example, an automated peptide synthesis apparatus (see, *e.g.,* Applied Biosystems, Foster City, CA).

In accordance with the invention, there are provided isolated or purified nucleic acids encoding glycoproteins denoted as SAM-6 Receptor (SAM-6/R) or SAM-6/R glycoprotein, having an apparent molecular weight in a range of about 80-82 kilodaltons (kDa) as determined by denaturing gel electrophoresis. In one embodiment, a nucleic acid sequence encodes a SAM-6/R glycoprotein having polypeptide sequence homology to Grp78 as set forth in SEQ ID NO:1. In another embodiment, a nucleic acid sequence encodes a SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct

from Grp78. In a further embodiment, a nucleic acid sequence encodes a SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety that is an epitope or is a part of an epitope to which SAM-6 antibody specifically binds. Nucleic acids according to the invention therefore include sequences encoding 1) SAM-6/R glycoprotein that has polypeptide sequence identity to Grp78 as set forth in SEQ ID NO:1; 2) SAM-6/R glycoprotein sequences (*e.g.*, capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety) that SAM-6 is capable of specifically binding; and 3) SAM-6/R glycoprotein subsequences and fragments (*e.g.*, SEQ ID NOs:2-12).

In accordance with the invention, there are also provided isolated or purified nucleic acids encoding subsequences and fragments of SAM-6/R glycoprotein. In one embodiment a nucleic acid sequence encodes a SAM-6/R glycoprotein sequence capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety, the carbohydrate moiety optionally distinct from a carbohydrate moiety of Grp78. In particular aspects, the nucleic acid sequence has a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000, nucleotides, or any numerical value or range within or encompassing such lengths, and optionally encodes a SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from a carbohydrate moiety of Grp78.

The terms "nucleic acid" and "polynucleotide" and the like refer to at least two or more ribo- or deoxy-ribonucleic acid base pairs (nucleotides) that are linked through a phosphoester bond or equivalent. Nucleic acids include polynucleotides and polynucleosides. Nucleic acids include single, double or triplex, circular or linear, molecules. Exemplary nucleic acids include but are not limited to: RNA, DNA, cDNA, genomic nucleic acid, naturally occurring and non naturally occurring nucleic acid, *e.g.*, synthetic nucleic acid.

Nucleic acids can be of various lengths. Nucleic acid lengths typically range from about 20 nucleotides to 20 Kb, or any numerical value or range within or encompassing such lengths, 10 nucleotides to 10Kb, 1 to 5 Kb or less, 1000 to about 500 nucleotides or less in length. Nucleic acids can also be shorter, for example, 100 to about 500 nucleotides, or from about 12 to 25, 25 to 50, 50 to 100, 100 to 250, or about 250 to 500 nucleotides in length, or any numerical value or range or value within or encompassing such lengths. Shorter polynucleotides are commonly referred to as "oligonucleotides" or "probes" of single- or double-stranded DNA. However, there is no upper limit to the length of such oligonucleotides.

Polynucleotides include L- or D-forms and mixtures thereof, which additionally may be modified to be resistant to degradation when administered to a subject. Particular examples include 5' and 3' linkages resistant to endonucleases and exonucleases present in various tissues or fluids of a subject.

In another embodiment, the invention provides nucleic acids that hybridize to a nucleic acid that encodes all or a subsequence or fragment of SAM-6/R glycoprotein sequence, that is at least 75-90% complementary or homologous to the nucleic acid sequence that encodes all or a subsequence or fragment of SAM-6/R glycoprotein sequence. In one embodiment, the nucleic acid sequence has a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000, nucleotides, or any numerical value or range within or encompassing such lengths. In particular aspects, the nucleic acid sequence hybridizes to a nucleic acid sequence that encodes SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from Grp78.

The term "hybridize" and grammatical variations thereof refer to the binding between nucleic acid sequences. Hybridizing sequences will generally have more than about 50% homology (e.g., 50%, 60%, 70%, 80%, 90%, or more identity) to a nucleic acid that encodes an amino acid sequence of a reference (*e.g.,* SAM-6/R glycoprotein) sequence or a sequence complementary to a nucleic acid that encodes an amino acid sequence of a reference (*e.g.,* SAM-6/R glycoprotein) sequence. Hybridizing sequences that are 100% or fully complementary to a reference sequence, for example, to a nucleic acid that encodes an amino acid sequence of a reference (*e.g.,* SAM-6/R glycoprotein) sequence, exhibit 100% base pairing with no mismatches. The hybridization region between hybridizing sequences typically is at least about 12-15 nucleotides, 15-20 nucleotides, 20-30 nucleotides, 30-50 nucleotides, 50-100 nucleotides, 100 to 200 nucleotides or more, or any numerical value or range within or encompassing such lengths.

In accordance with the invention, there are further provided antisense polynucleotides, small interfering RNA, and ribozyme nucleic acid that specifically hybridize to the nucleic acid sequence encoding SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.,* oxLDL), VLDL, glycosylated or deglycosylated, or a portion thereof, or a sequence complementary to a nucleic acid that encodes SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.,* oxLDL), VLDL, glycosylated or deglycosylated, or a portion thereof. Antisense polynucleotides can have a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000 nucleotides, or any numerical value or range within or encompassing such lengths. In one embodiment, a nucleic acid sequence comprises an antisense polynucleotide that

specifically hybridizes to the nucleic acid sequence encoding a SAM-6/R glycoprotein capable of having linked thereto an N- or O-linked carbohydrate moiety distinct from a carbohydrate moiety of Grp78. In particular aspects, an antisense is at least 90% complementary or homologous to a nucleic acid sequence encoding SAM-6/R glycoprotein, or a nucleic acid sequence encoding SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from a carbohydrate moiety of Grp78, or a sequence complementary to a nucleic acid encoding SAM-6/R glycoprotein, or a nucleic acid sequence encoding SAM-6/R glycoprotein capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from a carbohydrate moiety of Grp78

As used herein, the term "antisense" refers to a polynucleotide or peptide nucleic acid capable of binding to a specific DNA or RNA sequence. Antisense includes single, double, triple or greater stranded RNA and DNA polynucleotides and peptide nucleic acids (PNAs) that bind RNA transcript or DNA. Particular examples include RNA and DNA antisense that binds to sense RNA. For example, a single stranded nucleic acid can target a protein transcript that participates in metabolism, catabolism, removal or degradation of glycogen from a cell (*e.g.*, mRNA). Antisense molecules are typically 95-100% complementary to the sense strand but can be "partially" complementary, in which only some of the nucleotides bind to the sense molecule (less than 100% complementary, *e.g.*, 95%, 90%, 80%, 70% and sometimes less), or any numerical value or range within or encompassing such percent values.

Triplex forming antisense can bind to double strand DNA thereby inhibiting transcription of the gene. Oligonucleotides derived from the transcription initiation site of the gene, *e.g.*, between positions -10 and +10 from the start site, are one particular example.

Short interfering RNA (referred to as siRNA or RNAi) for inhibiting gene expression is known in the art (see, *e.g.*, Kennerdell *et al.*, Cell 95:1017 (1998); Fire *et al.*, Nature, 391:806 (1998); WO 02/44321; WO 01/68836; WO 00/44895, WO 99/32619, WO 01/75164, WO 01/92513, WO 01/29058, WO 01/89304, WO 02/16620; and WO 02/29858). RNAi silencing can be induced by a nucleic acid encoding an RNA that forms a "hairpin" structure or by expressing RNA from each end of an encoding nucleic acid, making two RNA molecules that hybridize.

Ribozymes, which are enzymatic RNA molecules that catalyze the specific cleavage of RNA can be used to inhibit expression of the encoded protein. Ribozymes form sequence-specific hybrids with complementary target RNA, which is then cleaved. Specific examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze

endonucleolytic cleavage of sequences encoding a protein that participates in metabolism, catabolism, removal or degradation of glycogen, for example.

Antisense, ribozymes, RNAi and triplex forming nucleic acid are referred to collectively herein as "inhibitory nucleic acid" or "inhibitory polynucleotides." Such inhibitory nucleic acid or polynucleotides can inhibit or prevent expression of SAM-6/R glycoprotein.

Inhibitory polynucleotides do not require expression control elements in order to function *in vivo*. Inhibitory polynucleotides can be absorbed by the cell or enter the cell via passive diffusion. Inhibitory polynucleotides can optionally be introduced into a cell using a vector. Inhibitory polynucleotides may be encoded by a nucleic acid so that it is transcribed. Furthermore, a nucleic acid encoding an inhibitory polynucleotide may be operatively linked to an expression control element for sustained or increased expression of the encoded antisense in cells or *in vivo*. Inhibitory nucleic acid can be designed based upon protein and nucleic acid sequences disclosed herein or available in the database.

Nucleic acid sequences further include nucleotide and nucleoside substitutions, additions and deletions, as well as derivatized forms and fusion/chimeric sequences (*e.g.*, encoding recombinant polypeptide). For example, due to the degeneracy of the genetic code, nucleic acids include sequences and subsequences degenerate with respect to nucleic acids that encode SAM-6/R glycoprotein and subsequences or fragments (*e.g.*, a SAM-6/R glycoprotein fragment capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety distinct from Grp78) or variants thereof. Other examples are nucleic acids complementary to a sequence that encodes an amino acid sequence of a SAM-6/R glycoprotein and subsequences or fragments thereof.

Nucleic acid deletions (subsequences and fragments) can have from about 10 to 25, 25 to 50 or 50 to 100 nucleotides. Such nucleic acids are useful for expressing polypeptide subsequences, for genetic manipulation (as primers and templates for PCR amplification), and as probes to detect the presence or an amount of a sequence encoding a protein (*e.g.*, via hybridization), in a cell, culture medium, biological sample (*e.g.*, tissue, organ, blood or serum), or in a subject.

Nucleic acids can be produced using various standard cloning and chemical synthesis techniques. Techniques include, but are not limited to nucleic acid amplification, *e.g.*, polymerase chain reaction (PCR), with genomic DNA or cDNA targets using primers (*e.g.*, a degenerate primer mixture) capable of annealing to antibody encoding sequence. Nucleic acids

can also be produced by chemical synthesis (*e.g.*, solid phase phosphoramidite synthesis) or transcription from a gene. The sequences produced can then be translated in vitro, or cloned into a plasmid and propagated and then expressed in a cell (*e.g.*, a host cell such as yeast or bacteria, a eukaryote such as an animal or mammalian cell or in a plant).

In accordance with the invention, there are further provided vectors that comprise nucleic acid sequences of the invention. In one embodiment, a vector includes a nucleic acid sequence encoding SAM-6/R glycoprotein. In another embodiment, a vector includes a nucleic acid sequence encoding SAM-6/R glycoprotein subsequence or fragment capable of having linked thereto at least one nitrogen (N)- or oxygen (O)-linked carbohydrate moiety that SAM-6 antibody is capable of specifically binding.

Vectors include viral, prokaryotic (bacterial) and eukaryotic (plant, fungal, mammalian) vectors. Vectors can be used for expression of nucleic acids in vitro or *in vivo*. Such vectors, referred to as "expression vectors," are useful for introducing nucleic acids, including nucleic acids that encode a SAM-6/R glycoprotein, subsequences and fragments thereof, nucleic acids that encode inhibitory nucleic acid, and expressing the encoded protein or inhibitory nucleic acid (*e.g.*, in solution or in solid phase), in cells or in a subject *in vivo*.

Vectors can also be used for manipulation of nucleic acids. For genetic manipulation "cloning vectors" can be employed, and to transcribe or translate the inserted nucleic acid.

A vector generally contains an origin of replication for propagation in a cell in vitro or *in vivo*. Control elements, including expression control elements, present within a vector, can be included to facilitate transcription and translation, as appropriate.

Vectors can include a selection marker. A "selection marker" is a gene that allows for the selection of cells containing the gene. "Positive selection" refers to a process in which cells that contain the selection marker survive upon exposure to the positive selection. Drug resistance is one example of a positive selection marker-cells containing the marker will survive in culture medium containing the selection drug, and cells lacking the marker will die. Selection markers include drug resistance genes such as *neo*, which confers resistance to G418; *hygr*, which confers resistance to hygromycin; and *puro*, which confers resistance to puromycin. Other positive selection marker genes include genes that allow identification or screening of cells containing the marker. These genes include genes for fluorescent proteins (GFP and GFP-like chromophores, luciferase), the lacZ gene, the alkaline phosphatase gene, and surface markers such as CD8, among others. "Negative selection" refers to a process in which cells containing a negative

selection marker are killed upon exposure to an appropriate negative selection agent. For example, cells which contain the herpes simplex virus-thymidine kinase (*HSV-tk*) gene (Wigler *et al.*, Cell 11:223 (1977)) are sensitive to the drug gancyclovir (GANC). Similarly, the *gpt* gene renders cells sensitive to 6-thioxanthine.

Viral vectors include those based upon retroviral (lentivirus for infecting dividing as well as non-dividing cells), foamy viruses (U.S. Patent Nos. 5,624,820, 5,693,508, 5,665,577, 6,013,516 and 5,674,703; WO92/05266 and WO92/14829), adenovirus (U.S. Patent Nos. 5,700,470, 5,731,172 and 5,928,944), adeno-associated virus (AAV) (U.S. Patent No. 5,604,090), herpes simplex virus vectors (U.S. Patent No. 5,501,979), cytomegalovirus (CMV) based vectors (U.S. Patent No. 5,561,063), reovirus, rotavirus genomes, simian virus 40 (SV40) or papilloma virus (Cone *et al.*, Proc. Natl. Acad. Sci. USA 81:6349 (1984); Eukaryotic Viral Vectors, Cold Spring Harbor Laboratory, Gluzman ed., 1982; Sarver *et al.*, Mol. Cell. Biol. 1:486 (1981); U.S. Patent No. 5,719,054). Adenovirus efficiently infects slowly replicating and/or terminally differentiated cells and can be used to target slowly replicating and/or terminally differentiated cells. Additional viral vectors useful for expression include parvovirus, Norwalk virus, coronaviruses, paramyxo- and rhabdoviruses, togavirus (*e.g.*, sindbis virus and semliki forest virus) and vesicular stomatitis virus (VSV).

Vectors including a nucleic acid can be expressed when the nucleic acid is operably linked to an expression control element. As used herein, the term "operably linked" refers to a physical or a functional relationship between the elements referred to that permit them to operate in their intended fashion. Thus, an expression control element "operably linked" to a nucleic acid means that the control element modulates nucleic acid transcription and as appropriate, translation of the transcript.

The term "expression control element" refers to nucleic acid that influences expression of an operably linked nucleic acid. Promoters and enhancers are particular non-limiting examples of expression control elements. A "promoter sequence" is a DNA regulatory region capable of initiating transcription of a downstream (3' direction) sequence. The promoter sequence includes nucleotides that facilitate transcription initiation. Enhancers also regulate gene expression, but can function at a distance from the transcription start site of the gene to which it is operably linked. Enhancers function at either 5' or 3' ends of the gene, as well as within the gene (*e.g.*, in introns or coding sequences). Additional expression control elements include leader sequences and fusion partner sequences, internal ribosome binding sites (IRES) elements for the creation of multigene, or polycistronic, messages, splicing signal for introns, maintenance of the correct

reading frame of the gene to permit in-frame translation of mRNA, polyadenylation signal to provide proper polyadenylation of the transcript of interest, and stop codons.

Expression control elements include "constitutive" elements in which transcription of an operably linked nucleic acid occurs without the presence of a signal or stimuli. Expression control elements that confer expression in response to a signal or stimuli, which either increase or decrease expression of operably linked nucleic acid, are "regulatable." A regulatable element that increases expression of operably linked nucleic acid in response to a signal or stimuli is referred to as an "inducible element." A regulatable element that decreases expression of the operably linked nucleic acid in response to a signal or stimuli is referred to as a "repressible element" (*i.e.*, the signal decreases expression; when the signal is removed or absent, expression is increased).

Expression control elements include elements active in a particular tissue or cell type, referred to as "tissue-specific expression control elements." Tissue-specific expression control elements are typically more active in specific cell or tissue types because they are recognized by transcriptional activator proteins, or other transcription regulators active in the specific cell or tissue type, as compared to other cell or tissue types.

Tissue-specific expression control elements include promoters and enhancers active in hyperproliferative cells, such as cell proliferative disorders including neoplasias, tumors and cancers, and metastasis. Particular non-limiting examples of such promoters are hexokinase II, COX-2, alpha-fetoprotein, carcinoembryonic antigen, DE3/MUC1, prostate specific antigen, C-erB2/neu, telomerase reverse transcriptase and hypoxia-responsive promoter.

For bacterial expression, constitutive promoters include T7, as well as inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter). In insect cell systems, constitutive or inducible promoters (*e.g.*, ecdysone) may be used. In yeast, constitutive promoters include, for example, ADH or LEU2 and inducible promoters such as GAL (see, *e.g.*, Ausubel *et al.*, In: Current Protocols in Molecular Biology, Vol. 2, Ch. 13, ed., Greene Publish. Assoc. & Wiley Interscience, 1988; Grant *et al.*, In: Methods in Enzymology, 153:516-544 (1987), eds. Wu & Grossman, 1987, Acad. Press, N.Y.; Glover, DNA Cloning, Vol. II, Ch. 3, IRL Press, Wash., D.C., 1986; Bitter, In: Methods in Enzymology, 152:673-684 (1987), eds. Berger & Kimmel, Acad. Press, N.Y.; and, Strathern *et al.*, The Molecular Biology of the Yeast Saccharomyces eds. Cold Spring Harbor Press, Vols. I and II (1982)).

For mammalian expression, constitutive promoters of viral or other origins may be used. For example, SV40, or viral long terminal repeats (LTRs) and the like, or inducible promoters

derived from the genome of mammalian cells (*e.g.*, metallothionein IIA promoter; heat shock promoter, steroid/thyroid hormone/retinoic acid response elements) or from mammalian viruses (*e.g.*, the adenovirus late promoter; mouse mammary tumor virus LTR) are used.

In accordance with the invention, there are provided host cells transformed or transfected with a nucleic acid or vector of the invention. Host cells include but are not limited to prokaryotic and eukaryotic cells such as bacteria, fungi (yeast), plant, insect, and animal (*e.g.*, mammalian, including primate and human) cells. For example, bacteria transformed with recombinant bacteriophage nucleic acid, plasmid nucleic acid or cosmid nucleic acid expression vectors; yeast transformed with recombinant yeast expression vectors; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid); insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus); and animal cell systems infected with recombinant virus expression vectors (*e.g.*, retroviruses, adenovirus, vaccinia virus), or transformed animal cell systems engineered for stable expression.

The cells may be a primary cell isolate, cell culture (*e.g.*, passaged, established or immortalized cell line), or part of a plurality of cells, or a tissue or organ *ex vivo* or in a subject (*in vivo*). In particular embodiments, a cell is a hyperproliferative cell, a cell comprising a cellular hyperproliferative disorder, an immortalized cell, neoplastic cell, tumor cell or cancer cell, or metastasis cell.

The term "transformed" or "transfected" when use in reference to a cell (*e.g.*, a host cell) or organism, means a genetic change in a cell following incorporation of an exogenous molecule, for example, a protein or nucleic acid (*e.g.*, a transgene) into the cell. Thus, a "transfected" or "transformed" cell is a cell into which, or a progeny thereof in which an exogenous molecule has been introduced by the hand of man, for example, by recombinant DNA techniques.

The nucleic acid or protein can be stably or transiently transfected or transformed (expressed) in the cell and progeny thereof. The cell(s) can be propagated and the introduced protein expressed, or nucleic acid transcribed. A progeny of a transfected or transformed cell may not be identical to the parent cell, since there may be mutations that occur during replication.

Typically, cell transfection or transformation employs a "vector," which refers to a plasmid, virus, such as a viral vector, or other vehicle known in the art that can be manipulated by insertion or incorporation of a nucleic acid.

A viral particle or vesicle can be designed to be targeted to particular cell types (*e.g.*, hyperproliferating cells) by inclusion of a protein on the surface that binds to a target cell ligand or receptor. Alternatively, a cell type-specific promoter and/or enhancer can be included in the vector in order to express the nucleic acid in target cells. Thus, the viral particle or vesicle itself, viral vector, or a protein on the viral surface can be made to target cells for transfection or transformation in vitro, *ex vivo* or *in vivo*.

Introduction of compositions (*e.g.*, protein and nucleic acid) into target cells (*e.g.*, host cells) can also be carried out by methods known in the art such as osmotic shock (*e.g.*, calcium phosphate), electroporation, microinjection, cell fusion, etc. Introduction of nucleic acid and polypeptide *in vitro*, *ex vivo* and *in vivo* can also be accomplished using other techniques. For example, a polymeric substance, such as polyesters, polyamine acids, hydrogel, polyvinyl pyrrolidone, ethylene-vinylacetate, methylcellulose, carboxymethylcellulose, protamine sulfate, or lactide/glycolide copolymers, polylactide/glycolide copolymers, or ethylenevinylacetate copolymers. A nucleic acid can be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization, for example, by the use of hydroxymethylcellulose or gelatin-microcapsules, or poly (methylmethacrolate) microcapsules, respectively, or in a colloid system. Colloidal dispersion systems include macromolecule complexes, nano-capsules, microspheres, beads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

Liposomes for introducing various compositions into cells are known in the art and include, for example, phosphatidylcholine, phosphatidylserine, lipofectin and DOTAP (*e.g.*, U.S. Patent Nos. 4,844,904, 5,000,959, 4,863,740, and 4,975,282; and GIBCO-BRL, Gaithersburg, Md). Piperazine based amphilic cationic lipids useful for gene therapy also are known (see, *e.g.*, U.S. Patent No. 5,861,397). Cationic lipid systems also are known (see, *e.g.*, U.S. Patent No. 5,459,127). Polymeric substances, microcapsules and colloidal dispersion systems such as liposomes are collectively referred to herein as "vesicles." Accordingly, viral and non-viral vector means of delivery into cells, tissue or organs, *in vitro*, *in vivo* and *ex vivo* are included.

The invention includes *in vivo* methods. For example, a cell such as a hyperproliferative cell or cellular hyperproliferative disorder that expresses SAM-6/R glycoprotein can be present in a subject, such as a mammal (*e.g.*, a human subject). Cells comprising the cell proliferative or cellular hyperproliferative disorder may therefore be treated by administering, for example, an antibody, or subsequence or fragment thereof, that specifically binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a

subsequence thereof, or an inhibitory nucleic acid thereof. Cells comprising the cell proliferative or cellular hyperproliferative disorder may also be treated by administering, for example, a SAM-6/R glycoprotein or a subsequence thereof, which can elicit an immune response against SAM-6/R glycoprotein thereby functioning as a vaccine. In addition, disorders and diseases associated with or caused by undesirable or excessive as SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, levels can be treated in accordance with the invention by administering, for example, an antibody, or subsequence or fragment thereof that specifically binds to SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, which reducesVLDL, LDL or oxLDL.

In accordance with the invention, there are provided methods of treating cell proliferation or a cell proliferative or cellular hyperproliferative disorder in a subject. In one embodiment, a method includes administering to a subject an antibody that specifically binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, in an amount effective to treat the cell proliferation or a cell proliferative or cellular hyperproliferative disorder in the subject. In another embodiment, a method includes administering to a subject a SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, in an amount effective to treat the cell proliferation or a cell proliferative or cellular hyperproliferative disorder in the subject.

As used herein, the terms "cell proliferative disorder" and "cellular hyperproliferative disorder" and grammatical variations thereof, when used in reference to a cell, tissue or organ, refers to any undesirable, excessive or abnormal cell, tissue or organ growth, proliferation, differentiation or survival. A hyperproliferative cell denotes a cell whose growth, proliferation, or survival is greater than desired, such as a reference normal cell, *e.g.*, a cell that is of the same tissue or organ but is not a hyperproliferative cell, or a cell that fails to differentiate normally. Cell proliferative and hyperproliferative disorders include diseases and physiological conditions, both benign hyperplastic conditions characterized by undesirable, excessive or abnormal cell numbers, cell growth, cell proliferation, cell survival or differentiation in a subject. Specific examples of such disorders include metastatic and non-metastatic neoplasia, tumors and cancers (malignancies).

In various embodiments, a method includes administering to a subject an antibody or subsequence thereof that specifically binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, in an amount effective to treat the cell proliferative or cellular hyperproliferative disorder in the

subject. In particular aspects, the disorder is a neoplasia, tumor or metastatic or non-metastatic cancer (malignancy). In additional aspects, the disorder affects or is present in part at least in breast, lung, thyroid, head and neck, nasopharynx, nose or sinuses, brain, spine, adrenal gland, thyroid, lymph, gastrointestinal (mouth, esophagus, stomach, duodenum, ileum, jejunum (small intestine), colon, rectum), genito-urinary tract (uterus, ovary, cervix, bladder, testicle, penis, prostate), kidney, pancreas, adrenal gland, liver, bone, bone marrow, lymph, blood, muscle, skin, or the hematopoetic system.

The terms "neoplasia" and "tumor" are used interchangeably herein and refer to a cell or population of cells of any cell, tissue or organ origin, whose growth, proliferation or survival is greater than growth, proliferation or survival of a normal counterpart cell. A "cancer" is a malignant neoplasia or tumor, which typically invades other regions, tissues or organs and has the potential to metastasize to other sites via blood or lymph transport.

Neoplasias, tumors and cancers include a sarcoma, carcinoma, adenocarcinoma, melanoma, myeloma, blastoma, glioma, lymphoma or leukemia. Exemplary cancers include, for example, carcinoma, sarcoma, adenocarcinoma, melanoma, neural (blastoma, glioma), mesothelioma and reticuloendothelial, lymphatic or haematopoietic neoplastic disorders (*e.g.*, myeloma, lymphoma or leukemia). In particular aspects, a neoplasia, tumor or cancer includes a lung adenocarcinoma, lung carcinoma, diffuse or interstitial gastric carcinoma, colon adenocarcinoma, prostate adenocarcinoma, esophagus carcinoma, breast carcinoma, pancreas adenocarcinoma, ovarian adenocarcinoma, or uterine adenocarcinoma.

Neoplasia, tumors and cancers include benign, malignant, metastatic and non-metastatic types, and include any stage (I, II, III, IV or V) or grade (G1, G2, G3, etc.) of neoplasia, tumor, or cancer, or a neoplasia, tumor, cancer or metastasis that is progressing, worsening, stabilized or in remission.

Neoplasias, tumors and cancers can arise from a multitude of primary tumor types, including but not limited to breast, lung, thyroid, head and neck, nasopharynx, nose or sinuses, brain, spine, adrenal gland, thyroid, lymph, gastrointestinal (mouth, esophagus, stomach, duodenum, ileum, jejunum (small intestine), colon, rectum), genito-urinary tract (uterus, ovary, cervix, bladder, testicle, penis, prostate), kidney, pancreas, adrenal gland, liver, bone, bone marrow, lymph, blood, muscle, skin, and the hematopoetic system, and may metastasize to secondary sites.

A "solid neoplasia, tumor or cancer" refers to neoplasia, tumor or cancer (*e.g.*, metastasis) that typically aggregates together and forms a mass. Specific examples include visceral tumors such as melanomas, breast, pancreatic, uterine and ovarian cancers, testicular cancer, including seminomas, gastric or colon cancer, hepatomas, adrenal, renal and bladder carcinomas, lung, head and neck cancers and brain tumors/cancers.

Carcinomas refer to malignancies of epithelial or endocrine tissue, and include respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. The term also includes carcinosarcomas, *e.g.*, which include malignant tumors composed of carcinomatous and sarcomatous tissues. Adenocarcinoma includes a carcinoma of a glandular tissue, or in which the tumor forms a gland like structure. Melanoma refers to malignant tumors of melanocytes and other cells derived from pigment cell origin that may arise in the skin, the eye (including retina), or other regions of the body. Additional carcinomas can form from the uterine/cervix, lung, head/neck, colon, pancreas, testes, adrenal gland, kidney, esophagus, stomach, liver and ovary.

Sarcomas refer to malignant tumors of mesenchymal cell origin. Exemplary sarcomas include for example, lymphosarcoma, liposarcoma, osteosarcoma, chondrosarcoma, leiomyosarcoma, rhabdomyosarcoma and fibrosarcoma.

Neural neoplasias include glioma, glioblastoma, meningioma, neuroblastoma, retinoblastoma, astrocytoma, oligodendrocytoma

Specific non-limiting examples of neoplasias, tumors and cancers amenable to treatment include malignant and non-malignant neoplasias, tumors and cancers, and metastasis. In particular, melanomas, gastric tissue, lung squamous cell carcinoma, lung adenocarcinoma cell and nasal cancer cells, of any stage (e.g., stages IA, IB, IIA, IIB, IIIA, IIIB or IV) or grade (e.g., grades G1, G2 or G3). Specific non-limiting examples of metastasis include lung squamous cell carcinoma and adenocarcinoma metastasis to lymph node and brain; breast cancer (invasive ductal) metastasis to lymph node; colon adenocarcinoma metastasis to liver and lymph node; SAM-6/R glycoprotein was detected on stomach adenocarcinoma (intestinal and diffuse) metastasis to lymph node; pancreas adenocarcinmoa metastasis to lymph node; head and neck squamous cell carcinoma metastasis to lymph node; and melanoma metastasis to rectum, esophagus, skin, parotid gland, colon, adrenal gland and nasal epithelium.

A "liquid neoplasia, tumor or cancer" refers to a neoplasia, tumor or cancer of the reticuloendothelial or hematopoetic system, such as a lymphoma, myeloma, or leukemia, or a neoplasia that is diffuse in nature. Particular examples of leukemias include acute and chronic lymphoblastic, myeolblastic and multiple myeloma. Typically, such diseases arise from poorly differentiated acute leukemias, *e.g.*, erythroblastic leukemia and acute megakaryoblastic leukemia. Specific myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML); lymphoid malignancies include, but are not limited to, acute lymphoblastic leukemia (ALL), which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Specific malignant lymphomas include, non-Hodgkin lymphoma and variants, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

In accordance with the invention, there are provided methods of reducing VLDL, LDL or oxLDL, as well as methods of treating disorders and diseases associated with or caused by undesirable or excessive VLDL, LDL or oxLDL levels. In one embodiment, a method includes administering to a subject an antibody that specifically binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL, in an amount effective to treat the disorder or disease associated with or caused by undesirable or excessive VLDL, LDL or oxLDL levels (*e.g.*, plasma levels) in the subject.

Non-limiting exemplary disorders and diseases associated with undesirable or excessive VLDL, LDL or oxLDL levels include hyperlipidemia, hypercholesterolemia, arteriosclerosis, cardiovascular disease, coronary heart disease (CHD), stroke, glomerulonecrosis, high blood pressure and diabetes. Thus, in additional embodiments, a method includes administering to a subject an antibody that specifically binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, in an amount effective to treat hyperlipidemia, hypercholesterolemia, arteriosclerosis, cardiovascular disease, coronary heart disease (CHD), stroke, glomerulonecrosis, high blood pressure or diabetes.

As used herein, the terms "treat," "treating," "treatment" and grammatical variations thereof mean subjecting an individual patient to a protocol, regimen, process or remedy, in which it is desired to obtain a physiologic response or outcome in that patient. Since every treated

patient may not respond to a particular treatment protocol, regimen, process or remedy, treating does not require that the desired physiologic response or outcome be achieved in each and every patient or patient population. Accordingly, a given patient or patient population may fail to respond or respond inadequately to treatment.

Methods of the invention may be practiced by any mode of administration or by any route, systemic, regional and local administration. Exemplary administration routes include intravenous, intrarterial, intradermal, intramuscular, subcutaneous, intra-pleural, transdermal (topical), transmucosal, intra-cranial, intra-spinal, intra-ocular, rectal, oral (alimentary) and mucosal.

Methods of the invention include, among other things, methods that provide a detectable or measurable improvement in a condition of a given subject, such as alleviating or ameliorating one or more adverse (physical) symptoms or consequences associated with the presence of a cell proliferative or cellular hyperproliferative disorder, neoplasia, tumor or cancer, or metastasis, i.e., a therapeutic benefit or a beneficial effect.

A therapeutic benefit or beneficial effect is any objective or subjective, transient, temporary, or long-term improvement in the condition or pathology, or a reduction in onset, severity, duration or frequency of an adverse symptom associated with or caused by cell proliferation or a cellular hyperproliferative disorder such as a neoplasia, tumor or cancer, or metastasis. A satisfactory clinical endpoint of a treatment method in accordance with the invention is achieved, for example, when there is an incremental or a partial reduction in severity, duration or frequency of one or more associated pathologies, adverse symptoms or complications, or inhibition or reversal of one or more of the physiological, biochemical or cellular manifestations or characteristics of cell proliferation or a cellular hyperproliferative disorder such as a neoplasia, tumor or cancer, or metastasis. A therapeutic benefit or improvement therefore be a cure, such as destruction of target proliferating cells (*e.g.*, neoplasia, tumor or cancer, or metastasis) or ablation of one or more, most or all pathologies, adverse symptoms or complications associated with or caused by cell proliferation or the cellular hyperproliferative disorder such as a neoplasia, tumor or cancer, or metastasis. However, a therapeutic benefit or improvement need not be a cure or complete destruction of all target proliferating cells (*e.g.*, neoplasia, tumor or cancer, or metastasis) or ablation of all pathologies, adverse symptoms or complications associated with or caused by cell proliferation or the cellular hyperproliferative disorder such as a neoplasia, tumor or cancer, or metastasis. For example, partial destruction of a tumor or cancer cell mass, or a stabilization of the tumor or cancer mass, size or cell numbers by

inhibiting progression or worsening of the tumor or cancer, can reduce mortality and prolong lifespan even if only for a few days, weeks or months, even though a portion or the bulk of the tumor or cancer mass, size or cells remain.

Specific non-limiting examples of therapeutic benefit include a reduction in neoplasia, tumor or cancer, or metastasis volume (size or cell mass) or numbers of cells, inhibiting or preventing an increase in neoplasia, tumor or cancer volume (*e.g.,* stabilizing), slowing or inhibiting neoplasia, tumor or cancer progression, worsening or metastasis, stimulating, inducing or increasing neoplasia, tumor or cancer cell lysis or apoptosis or inhibiting neoplasia, tumor or cancer proliferation, growth or metastasis. An invention method may not take effect immediately. For example, treatment may be followed by an increase in the neoplasia, tumor or cancer cell numbers or mass, but over time eventual stabilization or reduction in tumor cell mass, size or numbers of cells in a given subject may subsequently occur after cell lysis or apoptosis of the neoplasia, tumor or cancer, or metastasis. Reduction of VLDL, LDL or oxLDL levels may take several days, weeks or even months following treatment.

Additional benefits include reducing VLDL, LDL or oxLDL, reducing or reversing narrowing of arteries or veins in a subject. Improvement in lipid profiles and increasing HDL levels are also nonlimiting examples of treatment benefits.

Additional adverse symptoms and complications associated with neoplasia, tumor, cancer and metastasis that can be inhibited, reduced, decreased, delayed or prevented include, for example, nausea, lack of appetite, lethargy, pain and discomfort. Thus, a partial or complete decrease or reduction in the severity, duration or frequency of an adverse symptom or complication associated with or caused by a cellular hyperproliferative disorder, an improvement in the subjects well being, such as increased energy, appetite, psychological well being, are all particular non-limiting examples of therapeutic benefit. A therapeutic benefit or improvement therefore can also include a subjective improvement in the quality of life of a treated subject.

In various embodiments, a method reduces or decreases neoplasia, tumor or cancer, or or metastasis volume, inhibits or prevents an increase in neoplasia, tumor or cancer volume, inhibits or delays neoplasia, tumor or cancer progression or worsening, stimulates neoplasia, tumor or cancer, or metastasis cell lysis or apoptosis, or inhibits, reduces, decreases or delays neoplasia, tumor or cancer proliferation or metastasis. In an additional embodiment, a method prolongs or extends lifespan of the subject. In a further embodiment, a method improves the quality of life of the subject.

Examination of a biopsied sample containing a neoplasia, tumor or cancer, or metastasis (*e.g.*, blood or tissue sample), can establish neoplastic, tumor or cancer cell volume or cell numbers, and therefore whether a reduction or stabilization in mass or numbers of neoplastic, tumor or cancer cells or inhibition of neoplasia, tumor or cancer cell proliferation, growth or survival (apoptosis) has occurred. For a solid neoplasia, tumor or cancer, invasive and non-invasive imaging methods can ascertain neoplasia, tumor or cancer size or volume. Examination of blood or serum, for example, for populations, numbers and types of cells (*e.g.*, hematopoetic cellular hyperproliferative disorders) can establish whether a reduction or stabilization in mass or numbers of neoplastic, tumor or cancer cells or inhibition of neoplastic, tumor or cancer proliferation, growth or survival (apoptosis) has occurred.

Invention compositions and methods can be combined with any other treatment or therapy that provides a desired effect. In particular, treatments and therapies that have been characterized as having an anti-cell proliferative activity or function are applicable. Exemplary treatments and therapies include anti-cell proliferative or immune enhancing agents or drugs. In the case of VLDL, LDL or oxLDL levels, additional treatments and therapies include VLDL, LDL or oxLDL lowering agents and drugs, such as statins. The treatments and therapies can be performed prior to, substantially contemporaneously with any other methods of the invention, for example, an anti-cell proliferative cellular hyperproliferative disorder (*e.g.*, a neoplasia, tumor or cancer, or metastasis) or LDL reducing treatment or therapy.

The invention therefore provides combination methods in which the methods of the invention are used in a combination with any therapeutic regimen, treatment protocol or composition, such as an anti-cell proliferative or VLDL or LDL reducing protocol, agent or drug set forth herein or known in the art. In one embodiment, a method includes administering an antibody or an inhibitory nucleic acid and an anti-cell proliferative or immune enhancing treatment, agent or drug. The anti-cell proliferative or immune enhancing treatment, agent or drug can be administered prior to, substantially contemporaneously with or following administration of antibody or an inhibitory nucleic acid. In another embodiment, a method includes administering an VLDL, LDL or oxLDL reducing treatment, agent or drug.

As used herein, an "anti-cell proliferative," "anti-neoplastic," "anti-tumor," or "anti-cancer" treatment, therapy, activity or effect means any therapy, treatment regimen, agent, drug, protocol or process that is useful in treating pathologies, adverse symptoms or complications associated with or caused by abnormal or undesirable cell proliferation (cell hyperproliferation), a cellular hyperproliferative disorder, neoplasia, tumor or cancer, or metastasis. Particular

therapies, treatment regimens, agents, drugs, protocol or processes can inhibit, decrease, slow, reduce, delay, or prevent cell proliferation, cell growth, cellular hyperproliferation, neoplastic, tumor, or cancer (malignant) growth, proliferation, survival or metastasis. Such treatments, therapies, regimens, protocols, agents and drugs, can operate by disrupting, reducing, inhibiting or delaying cell cycle progression or cell proliferation or growth; increasing, stimulating or enhancing cell apoptosis, lysis or death; inhibiting nucleic acid or protein synthesis or metabolism; reducing, decreasing, inhibiting or delaying cell division; or decreasing, reducing or inhibiting cell survival, or production or utilization of a cell survival factor, growth factor or signaling pathway (extracellular or intracellular).

Examples of anti-cell proliferative treatments and therapies include chemotherapy, immunotherapy, radiotherapy (ionizing or chemical), local or regional thermal (hyperthermia) therapy and surgical resection.

Specific non-limiting classes of anti-cell proliferative agents and drugs include alkylating agents, anti-metabolites, plant extracts, plant alkaloids, nitrosoureas, hormones (steroids), nucleoside and nucleotide analogues. Specific non-limiting examples of microbial toxins include bacterial cholera toxin, pertussis toxin, anthrax toxin, diphtheria toxin, and plant toxin ricin. Specific examples of drugs include cyclophosphamide, azathioprine, cyclosporin A, melphalan, chlorambucil, mechlorethamine, busulphan, methotrexate, 6-mercaptopurine, thioguanine, 5-fluorouracil, 5-fluorouridine, cytosine arabinoside, AZT, 5-azacytidine (5-AZC) and 5-azacytidine related compounds, bleomycin, actinomycin D, mithramycin, mitomycin C, carmustine, calicheamicin, lomustine, semustine, streptozotocin, teniposide, etoposide, hydroxyurea, cisplatin, carboplatin, levamisole, mitotane, procarbazine, dacarbazine, taxol, vinblastine, vincristine, vindesine, doxorubicin, daunomycin and dibromomannitol. Specific non-limiting examples of hormones include prednisone, prednisolone, diethylstilbesterol, flutamide, leuprolide, and gonatrophin releasing hormone antagonists.

Radiotherapy includes internal or external delivery to a subject. For example, alpha, beta, gamma and X-rays can administered to the subject externally without the subject internalizing or otherwise physically contacting the radioisotope. Specific examples of X-ray dosages range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 5/week), to single doses of 2000 to 6000 roentgens. Dosages vary widely, and depend on duration of exposure, the half-life of the isotope, the type of radiation emitted, the cell type and location treated and the progressive stage of the disease. Specific non-limiting examples of radionuclides include, for example, ^{47}Sc

^{67}Cu, ^{72}Se, ^{88}Y, ^{90}Sr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{105}Rh, ^{111}In, ^{125}I, ^{131}I, ^{149}Tb, ^{153}Sm, ^{186}Re, ^{188}Re, ^{194}Os, ^{203}Pb, ^{211}At, ^{212}Bi, ^{213}Bi, ^{212}Pb, ^{223}Ra, ^{225}Ac, ^{227}Ac, and ^{228}Th.

Antibodies that bind to tumor cells are a particular example of an anti-cell proliferative treatment or therapy. Anti-tumor antibodies include, for example, M195 antibody which binds to leukemia cell CD33 antigen (U.S. Patent No. 6,599,505); monoclonal antibody DS6 which binds to ovarian carcinoma CA6 tumor-associated antigen (U.S. Patent No. 6,596,503); human IBD12 monoclonal antibody which binds to epithelial cell surface H antigen (U.S. Patent No. 4,814,275); and BR96 antibody which binds to Le^x carbohydrate epitope expressed by colon, breast, ovary, and lung carcinomas. Additional anti-tumor antibodies that can be employed include, for example, Herceptin (anti-Her-2 neu antibody), Rituxan®, Zevalin, Bevacizumab (Avastin), Bexxar, Campath®, Oncolym, 17-1A (Edrecolomab), 3F8 (anti-neuroblastoma antibody), MDX-CTLA4, IMC-C225 (Cetuximab) and Mylotarg.

As used here, the term "immune enhancing," when used in reference to a treatment, therapy, agent or drug means that the treatment, therapy, agent or drug provides an increase, stimulation, induction or promotion of an immune response, humoral or cell-mediated. Such therapies can enhance immune response generally, or enhance immune response to a specific target, *e.g.*, a cell proliferative or cellular hyperproliferative disorder such as a neoplasia, tumor or cancer, or metastasis.

Specific non-limiting examples of immune enhancing agents include antibody, cell growth factors, cell survival factors, cell differentiative factors, cytokines and chemokines. Additional examples of immune enhancing agents and treatments include immune cells such as lymphocytes, plasma cells, macrophages, dendritic cells, NK cells and B-cells that either express antibody against the cell proliferative disorder or otherwise are likely to mount an immune response against the cell proliferative disorder. Cytokines that enhance or stimulate immunogenicity include IL-2, IL-1α, IL-1β, IL-3, IL-6, IL-7, granulocyte-macrophage-colony stimulating factor (GMCSF), IFN-γ, IL-12, TNF-α, and TNFβ, which are also non-limiting examples of immune enhancing agents. Chemokines including MIP-1α, MIP-1β, RANTES, SDF-1, MCP-1, MCP-2, MCP-3, MCP-4, eotaxin, eotaxin-2, I-309/TCA3, ATAC, HCC-1, HCC-2, HCC-3, PARC, TARC, LARC/MIP-3α, CKβ, CKβ6, CKβ7, CKβ8, CKβ9, CKβ11, CKβ12, C10, IL-8, ENA-78, GROα, GROβ, GCP-2, PBP/CTAPIIIβ-TG/NAP-2, Mig, PBSF/SDF-1, and lymphotactin are further non-limiting examples of immune enhancing agents.

Methods of the invention also include, among other things, methods that result in a reduced need or use of another treatment protocol or therapeutic regimen, process or remedy. For example, for a neoplasia, tumor or cancer, or metastasis, a method of the invention has a therapeutic benefit if in a given subject it results in a less frequent or reduced dose or elimination of an anti-cell proliferative (*e.g.*, anti-neoplastic, anti-tumor or anti-cancer) or immune enhancing treatment or therapy, such as a chemotherapeutic drug, radiotherapy, immunotherapy, or surgery for neoplasia, tumor or cancer, or metastasis treatment or therapy.

In accordance with the invention, methods of reducing need or use of an anti-cell proliferative (*e.g.*, anti-neoplastic, anti-tumor, anti-cancer or anti-metastasis) treatment or therapy are provided. In one embodiment, a method includes administering to a subject an antibody that binds to SAM-6/R glycoprotein in an amount effective to treat a cellular hyperproliferative disorder (*e.g.*, a neoplasia, tumor or cancer, or metastasis), and to reduce or eliminate need for an anti-cell proliferative (anti-neoplasia, anti-tumor or anti-cancer, or anti-metastasis) or immune-enhancing therapy. The methods can be performed prior to, substantially contemporaneously with or following administration of an anti-neoplastic, tumor, cancer or metastasis, or immune-enhancing therapy.

The doses or "amount effective" or "amount sufficient" in a method of treatment or therapy in which it is desired to achieve a therapeutic benefit or improvement includes, for example, any objective or subjective alleviation or amelioration of one, several or all pathologies, adverse symptoms or complications associated with or caused by the target (*e.g.*, cellular hyperproliferative disorder), to a measurable or detectable extent, although preventing, inhibiting or delaying a progression or worsening of the target (*e.g.*, cellular hyperproliferative disorder) pathology, adverse symptom or complication, is a satisfactory outcome. Thus, in the case of a cellular hyperproliferative disorder, the amount will be sufficient to provide a therapeutic benefit to a given subject or to alleviate or ameliorate a pathology, adverse symptom or complication of the disorder in a given subject. The dose may be proportionally increased or reduced as indicated by the status of treatment or therapeutic target (*e.g.*, cellular hyperproliferative disorder) or any side effect(s) of the treatment or therapy.

Exemplary non-limiting amounts (doses) are in a range of about 0.1 mg/kg to about 100 mg/kg, and any numerical value or range or value within such ranges. Greater or lesser amounts (doses) can be administered, for example, 0.01-500 mg/kg, and any numerical value or range or value within such ranges. Additional exemplary non-limiting amounts (doses) range from about

0.5-50 mg/kg, 1.0-25 mg/kg, 1.0-10 mg/kg, and any numerical value or range or value within such ranges.

Methods of the invention may be practiced one or more times (*e.g.*, 1-10, 1-5 or 1-3 times) per day, week, month, or year. The skilled artisan will know when it is appropriate to delay or discontinue administration. An exemplary non-limiting dosage schedule is 1-7 times per week, for 1, 23, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more weeks, and any numerical value or range or value within such ranges.

Of course, as is typical for any treatment or therapy, different subjects will exhibit different responses to treatment and some may not respond or respond inadequately to a particular treatment protocol, regimen or process. Amounts effective or sufficient will therefore depend at least in part upon the disorder treated (*e.g.*, cell proliferation, benign hyperplasia or a neoplasia, tumor or cancer and the type or stage, *e.g.*, the tumor or cancer grade and if it is advanced, late or early stage), the therapeutic effect desired, as well as the individual subject (*e.g.*, the bioavailability within the subject, gender, age, etc.) and the subject's response to the treatment based upon genetic and epigenetic variability (*e.g.*, pharmacogenomics).

Cell toxicity and viability (cell apoptosis, lysis, growth proliferation, etc.) can be measured in a variety of ways on the basis of colorimetric, luminescent, radiometric, or fluorometric assays known in the art. Colorimetric techniques for determining cell viability include, for example, Trypan Blue exclusion (see, for example, Examples 1 and 2). In brief, cells are stained with Trypan Blue and counted using a hemocytometer. Viable cells exclude the dye whereas dead and dying cells take up the blue dye and are easily distinguished under a light microscope. Neutral Red is adsorbed by viable cells and concentrates in cell lysosomes; viable cells can be determined with a light microscope by quantitating numbers of Neutral Red stained cells.

Fluorometric techniques for determining cell viability include, for example, propidium iodide, a fluorescent DNA intercalating agent. Propidium iodide is excluded from viable cells but stains the nucleus of dead cells. Flow cytometry of propidium iodide labeled cells can then be used to quantitate viable and dead cells. Release of lactate dehydrogenase (LDH) indicates structural damage and death of cells, and can be measured by a spectrophotometric enzyme assay. Bromodeoxyuridine (BrdU) is incorporated into newly synthesized DNA and can be detected with a fluorochrome-labeled antibody. The fluorescent dye Hoechst 33258 labels DNA and can be used to quantitate proliferation of cells (*e.g.*, flow cytometry). Quantitative incorporation of the fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE or CFDA-SE) can

provide cell division analysis (*e.g.*, flow cytometry). This technique can be used either in vitro or *in vivo*. 7-aminoactinomycin D (7-AAD) is a fluorescent intercalator that undergoes a spectral shift upon association with DNA, and can provide cell division analysis (*e.g.*, flow cytometry).

Radiometric techniques for determining cell proliferation include, for example, [^{3}H]-Thymidine, which is incorporated into newly synthesized DNA of living cells and frequently used to determine proliferation of cells. Chromium (^{51}Cr)-release from dead cells can be quantitated by scintillation counting in order to quantitate cell viability.

Luminescent techniques for determining cell viability include, for example, the CellTiter-Glo luminescent cell viability assay (Promega Madison WI). This technique quantifies the amount of ATP present to determine the number of viable cells.

Commercially available kits for determining cell viability and cell proliferation include, for example, Cell Proliferation Biotrak ELISA (Amersham Biosciences Piscataway, NJ); the Guava ViaCount™ Assay, which provides rapid cell counts and viability determination based on differential uptake of fluorescent reagents (Guava Technologies, Hayward, CA); the CyQUANT® Cell Proliferation Assay Kit (Molecular Probes, Inc., Eugene, OR); and the CytoLux Assay Kit (PerkinElmer Life Sciences Inc., Boston, MA). The DELFIA® Assay Kits (PerkinElmer Life Sciences Inc., Boston, MA) can determine cell proliferation and viability using a time-resolved fluorometric method. The Quantos™ Cell Proliferation Assay is a fluorescence-based assay that measures the fluorescence of a DNA-dye complex from lysed cells (Stratagene, La Jolla, CA). The CellTiter-Glo cell viability assay is a luminescent assay for measuring cell viability (Promega, Madison WI).

The terms "subject" and "patient" are used interchangeably herein and refer to animals, typically mammals, such as humans, non-human primates (gorilla, chimpanzee, orangutan, macaque, gibbon), domestic animals (dog and cat), farm and ranch animals (horse, cow, goat, sheep, pig), laboratory and experimental animals (mouse, rat, rabbit, guinea pig). Subjects include disease model animals (*e.g.*, such as mice, rats and non-human primates) for studying *in vivo* efficacy (*e.g.*, a neoplasia, tumor or cancer, or metastasis animal model). Human subjects include children, for example, newborns, infants, toddlers and teens, between the ages of 1 and 5, 5 and 10 and 10 and 18 years, adults between the ages of 18 and 60 years, and the elderly, for example, between the ages of 60 and 65, 65 and 70 and 70 and 100 years.

Subjects include mammals (*e.g.*, humans) in need of treatment, that is, they have undesirable or aberrant cell proliferation (cell hyperproliferation) or a cellular hyperproliferative

disorder. Subjects include those at risk of having a cellular hyperproliferative disorder (*e.g.*, exhibit undesirable cell proliferation that is predisposed to become a cellular hyperproliferative disorder), a candidate subject for or a subject in need of an anti-cell proliferative or immune enhancing treatment or therapy due to a lab or clinical diagnosis warranting such treatment, subjects undergoing an anti-cell proliferative or immune enhancing therapy, and subjects having undergone an anti-cell proliferative or immune enhancing therapy and are at risk of relapse or recurrence.

At risk subjects include those with a family history, genetic predisposition, or who have suffered a previous affliction with a cell proliferative or cellular hyperproliferative disorder (*e.g.*, a benign hyperplasia, neoplasia, tumor or cancer, or metastasis), and are at risk of relapse or recurrence. At risk subjects further include environmental exposure to carcinogens or mutagens, such as smokers, or those in an occupational (industrial, chemical, agricultural) setting. Such subjects at risk for developing a cell proliferative or cellular hyperproliferative disorder such as neoplasia, tumor or cancer can be identified with genetic screens for tumor associated genes, gene deletions or gene mutations. Subjects that lack *Brca1* are at risk for developing breast cancer, for example. Subjects at risk for developing colon cancer have deleted or mutated tumor suppressor genes, such as adenomatous polyposis coli (*APC*), for example. At risk subjects having particular genetic predisposition towards cell proliferative disorders are known (see, *e.g.*, The Genetic Basis of Human Cancer 2nd ed. by Bert Vogelstein (Editor), Kenneth W. Kinzler (Editor) (2002) McGraw-Hill Professional; The Molecular Basis of Human Cancer. Edited by WB Coleman and GJ Tsongalis (2001) Humana Press; and The Molecular Basis of Cancer. Mendelsohn *et al.*, WB Saunders (1995)).

At risk subjects can therefore be treated in order to inhibit or reduce the likelihood of developing a cell proliferative or cellular hyperproliferative disorder, or after having been cured of a cell proliferative disorder, suffering a relapse or recurrence of the same or a different cell proliferative or cellular hyperproliferative disorder. The result of such treatment can be to reduce the risk of developing a cell proliferative or cellular hyperproliferative disorder, or to prevent a cell proliferative or cellular hyperproliferative disorder, or a pathology, adverse symptom or complication thereof in the treated at risk subject.

The invention further provides kits, including proteins (*e.g.*, antibodies), nucleic acids, agents, drugs and pharmaceutical formulations, packaged into suitable packaging material, optionally in combination with instructions for using the kit components, *e.g.*, instructions for performing a method of the invention. In one embodiment, a kit includes an antibody that binds

to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated or a subsequence thereof, and instructions for detecting one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof. In another embodiment, a kit includes an antibody that binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or an inhibitory nucleic acid and instructions for treating a condition treatable with an antibody or an inhibitory nucleic acid that binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof. In one aspect, the instructions are for treating undesirable cell proliferation or hyperproliferation, or a cellular hyperproliferative disorder. In another aspect, the instructions are for treating a neoplasia, tumor or cancer, or metastasis. In a further embodiment, a kit includes an antibody that binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, instructions for treating undesirable cell proliferation or hyperproliferation, or a cellular hyperproliferative disorder, and an anti-cell proliferative or immune enhancing treatment, agent or drug. In various aspects, a kit includes an anti-neoplastic, anti-cancer or anti-tumor agent. In still a further aspects, a kit includes an article of manufacture, for example, an article of manufacture for delivering the antibody or nucleic acid, anti-cell proliferative or immune enhancing treatment, agent or drug into a subject locally, regionally or systemically.

The term "packaging material" refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterilely, and can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampules, etc.). The label or packaging insert can include appropriate written instructions, for example, practicing a method of the invention, *e.g.*, treating a cell proliferative or cellular hyperproliferative disorder, an assay for screening for, detecting or identifying one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated, or a subsequence thereof, or a nucleic acid, etc. Thus, in additional embodiments, a kit includes a label or packaging insert including instructions for practicing a method of the invention in solution, in vitro, *in vivo*, or *ex vivo*.

Instructions can therefore include instructions for practicing any of the methods of the invention described herein. For example, invention pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration to a subject to treat a cell proliferative or cellular hyperproliferative disorder, such as a neoplasia, tumor or cancer, or metastasis. Instructions may additionally include indications of a satisfactory clinical endpoint or

any adverse symptoms or complications that may occur, storage information, expiration date, or any information required by regulatory agencies such as the Food and Drug Administration for use in a human subject.

The instructions may be on "printed matter," *e.g.,* on paper or cardboard within the kit, on a label affixed to the kit or packaging material, or attached to a vial or tube containing a component of the kit. Instructions may comprise voice or video tape and additionally be included on a computer readable medium, such as a disk (hard disk), optical CD such as CD- or DVD-ROM/RAM, electrical storage media such as FLASH, RAM and ROM and hybrids of these such as magnetic/optical storage media.

Invention kits can additionally include a buffering agent, a preservative, or a protein/nucleic acid stabilizing agent. The kit can also include control components for assaying for activity, *e.g.,* a control sample or a standard. Each component of the kit can be enclosed within an individual container or in a mixture and all of the various containers can be within single or multiple packages.

The proteins (*e.g.,* SAM-6/R glycoprotein), antibodies (*e.g.,* SAM-6/R glycoprotein antibody) nucleic acids, and other compositions and methods of the invention can be included in or employ pharmaceutical formulations. Such pharmaceutical formulations are useful for treatment of, or administration or delivery to, a subject *in vivo* or *ex vivo*.

Pharmaceutical formulations include "pharmaceutically acceptable" and "physiologically acceptable" carriers, diluents or excipients. As used herein the terms "pharmaceutically acceptable" and "physiologically acceptable" include solvents (aqueous or non-aqueous), solutions, emulsions, dispersion media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration. Such formulations can be contained in a liquid; emulsion, suspension, syrup or elixir, or solid form; tablet (coated or uncoated), capsule (hard or soft), powder, granule, crystal, or microbead. Supplementary compounds (*e.g.,* preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the formulations.

Pharmaceutical formulations can be made to be compatible with a particular local, regional or systemic administration or delivery route. Thus, pharmaceutical formulations include carriers, diluents, or excipients suitable for administration by particular routes. Specific non-limiting examples of routes of administration for compositions of the invention are parenteral, *e.g.,* intravenous, intrarterial, intradermal, intramuscular, subcutaneous, intra-pleural, transdermal

(topical), transmucosal, intra-cranial, intra-spinal, intra-ocular, rectal, oral (alimentary), mucosal administration, and any other formulation suitable for the treatment method or administration protocol.

Solutions or suspensions used for parenteral application can include: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide.

Pharmaceutical formulations for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Antibacterial and antifungal agents include, for example, parabens, chlorobutanol, phenol, ascorbic acid and thimerosal. Isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride can be included in the composition. Including an agent which delays absorption, for example, aluminum monostearate or gelatin can prolong absorption of injectable compositions.

Sterile injectable formulations can be prepared by incorporating the active composition in the required amount in an appropriate solvent with one or a combination of above ingredients. Generally, dispersions are prepared by incorporating the active composition into a sterile vehicle containing a basic dispersion medium and any other ingredient. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation include, for example, vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously prepared solution thereof.

For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives.

Transmucosal administration can be accomplished through the use of nasal sprays, inhalation devices (*e.g.*, aspirators) or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, creams or patches.

The pharmaceutical formulations can be prepared with carriers that protect against rapid elimination from the body, such as a controlled release formulation or a time delay material such as glyceryl monostearate or glyceryl stearate. The formulations can also be delivered using articles of manufacture such as implants and microencapsulated delivery systems to achieve local, regional or systemic delivery or controlled or sustained release.

Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations are known to those skilled in the art. The materials can also be obtained commercially from Alza Corporation (Palo Alto, CA). Liposomal suspensions (including liposomes targeted to cells or tissues using antibodies or viral coat proteins) can also be used as pharmaceutically acceptable carriers. These can be prepared according to known methods, for example, as described in U.S. Patent No. 4,522,811.

Additional pharmaceutical formulations appropriate for administration are known in the art (see, *e.g.*, Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20th ed., Lippincott, Williams & Wilkins (2000); Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th ed., Lippincott Williams & Wilkins Publishers (1999); Kibbe (ed.), Handbook of Pharmaceutical Excipients American Pharmaceutical Association, 3rd ed. (2000); and Remington's Pharmaceutical Principles of Solid Dosage Forms, Technonic Publishing Co., Inc., Lancaster, Pa., (1993)).

The compositions used in accordance with the invention, including proteins (antibodies), nucleic acid (inhibitory), treatments, therapies, agents, drugs and pharmaceutical formulations can be packaged in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages treatment; each unit contains a quantity of the composition in association with the carrier, excipient, diluent, or vehicle calculated to produce the desired treatment or therapeutic (*e.g.*, beneficial) effect. The unit dosage forms will depend on a variety of factors including, but not necessarily limited to, the particular composition employed, the effect to be achieved, and the pharmacodynamics and pharmacogenomics of the subject to be treated.

The invention provides cell-free (*e.g.*, in solution, in solid phase) and cell-based (*e.g.*, *in vitro* or *in vivo*) methods of screening, detecting and identifying one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, deglycosylated Grp78 or deglycosylated LDL. The methods can be performed in solution, *in vitro* using a biological material or sample, and *in vivo*, for example, using neoplastic, tumor or cancer, or metastasis cells, tissue or organ (*e.g.*, a biopsy) from an animal.

In accordance with the invention, there are provided methods of identifying, detecting or screening for one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, and methods for identifying, detecting or screening for a nucleic acid or portion thereof encoding a SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, sequence. In one embodiment, a method includes contacting a biological material or sample with an antibody that binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, under conditions allowing binding of the antibody; and assaying for binding of the antibody to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof. The binding of the antibody detects the presence of one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof. In another embodiment, a method includes contacting a biological material or sample with a polynucleotide that hybridizes to a nucleic acid or portion thereof encoding a SAM-6/R glycoprotein sequence under conditions allowing binding of the polynucleotide to the nucleic acid; and assaying for binding of the antibody to SAM-6/R glycoprotein. The binding of the polynucleotide to the nucleic acid detects the presence of SAM-6/R glycoprotein. In one aspect, the SAM-6/R glycoprotein is present on a cell or tissue. In another aspect, the biological material or sample is obtained from a mammalian subject. In a further aspect, the antibody that binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, is distinct from SAM-6 antibody, e.g., as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

The invention also provides cell-free (*e.g.*, in solution, in solid phase) and cell-based (*e.g.*, *in vitro* or *in vivo*) methods of diagnosing a subject having or at increased risk of having undesirable or aberrant cell proliferation or a cellular hyperproliferative disorder (*e.g.*, neoplasia, tumor or cancer, or metastasis). The methods can be performed in solution, *in vitro* using a

biological material or sample, for example, a biopsy of suspicious cells that may comprise or be indicative of neoplastic, tumor or cancer, or metastasis cells, tissue or organ, or serum, plasma, urine, saliva, menstruate, or feces. The methods can also be preformed *in vivo*, for example, in an animal.

In accordance with the invention, there are provided methods of diagnosing a subject having or at increased risk of having undesirable or aberrant cell proliferation or a cellular hyperproliferative disorder (*e.g.*, neoplasia, tumor or cancer, or metastasis). In one embodiment, a method includes contacting a biological material or sample from a subject with an antibody that binds to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, under conditions allowing binding of the antibody; and assaying for binding of the antibody to one or more of SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, or a subsequence thereof. The binding of the antibody to the SAM-6/R glycoprotein, Grp78, apoB100, LDL (*e.g.*, oxLDL), VLDL, glycosylated or deglycosylated forms thereof, or a subsequence thereof, diagnoses the subject as having or at increased risk of having undesirable or aberrant cell proliferation or a cellular hyperproliferative disorder (*e.g.*, neoplasia, tumor or cancer, or metastasis). In one aspect, the biological material or sample is obtained from a human. In another aspect, the biological material or sample comprises a biopsy (*e.g.*, a biopsy of lung, pancreas, stomach, breast, esophagus, ovary or uterus). In further aspects, the biological material or sample comprises serum, plasma, urine, saliva, menstruate, or feces.

Identifying, detecting, screening and diagnostic assays of the invention can be practiced by analysis of suspect hyperproliferating cells, for example, a cell of a cellular hyperproliferative disorder. Cells include hyperproliferating, immortalized, neoplastic, tumor and cancer cell lines and primary isolates derived from breast, lung, thyroid, head and neck, nasopharynx, nose or sinuses, brain, spine, adrenal gland, thyroid, lymph, gastrointestinal (mouth, esophagus, stomach, duodenum, ileum, jejunum (small intestine), colon, rectum), genito-urinary tract (uterus, ovary, cervix, bladder, testicle, penis, prostate), kidney, pancreas, adrenal gland, liver, bone, bone marrow, lymph, blood, muscle, skin, and the hematopoetic system, and metastasis or secondary sites.

The term "contacting," when used in reference to a composition such as a protein (*e.g.*, antibody), material, sample, or treatment, means a direct or indirect interaction between the composition (*e.g.*, protein such as an antibody) and the other referenced entity. A particular example of direct interaction is binding. A particular example of an indirect interaction is where

the composition acts upon an intermediary molecule, which in turn acts upon the referenced entity. Thus, for example, contacting a cell (*e.g.*, that comprises a cellular hyperproliferative disorder) with an antibody includes allowing the antibody to bind to the cell (*e.g.*, through binding to SAM-6/R glycoprotein), or allowing the antibody to act upon an intermediary that in turn acts upon the cell.

The terms "assaying" and "measuring" and grammatical variations thereof are used interchangeably herein and refer to either qualitative or quantitative determinations, or both qualitative and quantitative determinations. When the terms are used in reference to binding, any means of assessing the relative amount, affinity or specificity of binding is contemplated, including the various methods set forth herein and known in the art. For example, antibody binding can be assayed or measured by an ELISA assay.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention relates. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described herein.

U.S. Provisional Application Serial No. 61/151,149, and International Application Nos. PCT/EP/2004/012970 (publication WO 2005/047332 A1) and PCT/DE/2004/002503 (publication WO 2005/049635 A2) are expressly incorporated herein by reference in their entirety. All other publications, patents, Genbank accession numbers and other references cited herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

As used herein, singular forms "a", "and," and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a glycoprotein" or "antibody" includes a plurality of glycoproteins or antibodies and reference to "a treatment or therapy" can include multiple or sequential treatments or therapies, and so forth.

As used herein, all numerical values or numerical ranges include whole integers within or encompassing such ranges and fractions of the values or the integers within or encompassing ranges unless the context clearly indicates otherwise. Thus, for example, reference to a range of 90-100%, includes 91%, 92%, 93%, 94%, 95%, 95%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth. For example, reference to a range of 1-5,000 fold includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,

18, 19, 20, fold, etc., as well as 1.1, 1.2, 1.3, 1.4, 1.5, fold, etc., 2.1, 2.2, 2.3, 2.4, 2.5, fold, etc., and so forth. A reference to a range includes a reference to ssubranges with that range. Thus, for example, reference to a range of 90-100 includes a range of 91-99, 92-98, 93-95, 94-96, as well as 92-94, 93-95, 93-96, 95-97, 95-98, 95-99, 95-100, etc. A series of ranges include both lower and upper ends of thoes ranges combined into ranges. Thus, for example, reference to a series of ranges such as 50-100 100-200, and 200-300, includes a range of 50-200, 50-300, 100-300, etc.

The invention is generally disclosed herein using affirmative language to describe the numerous embodiments. The invention also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, procedures, assays or analysis. Thus, even though the invention is generally not expressed herein in terms of what the invention does not include, aspects that are not expressly included in the invention are nevertheless disclosed.

The following is a table of carbohydrate structures and abbreviations for those structures.

Table 1

Carbohydrate structure	Abbreviation
GalNAcβ1-4GlcNAcβ-	Lac-di-Nac
GlcNAcβ1-3Galβ-	GlcNacβ3Gal
GlcNAcβ1-6(GlcNAcβ1-3)Galβ1-4GlcNAcβ-	Tk
GalNAcβ1-4Galβ1-4Glcβ-	GA1
GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-	A type 2
Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ-	B type 2
Galα1-3Galβ1-4Glcβ-	Gala1-3'Lac
GlcNAcβ1-2Galβ1-3GalNAcα-	GlcNAcβ1-2'TF
Galα1-4GlcNAcβ-	Galα4GlcNAc
Neu5Acβ–	β-N-acetylneuraminic acid
Glcα1-4Glcβ-	maltose
Glcα-	α-D-glucose
Glcβ-	β-D-glucose
Galα-	α-D-galactose
Galβ-	β-D-galactose
α-D-Man-	α-D-mannose
6-H_2PO_3Manα-	α-D-mannose-6-phosphate
α-L-Fuc-	α-L-fucose
β-D-GlcNAc-	β-N-acetyl-D-glucosamine
α-D-GalNAc-	α-N-acetyl-D-glalactosamine (Tn)
β-D-GalNAc-	β-N-acetyl-D-glalactosamine
Manα1-3(Manα1-6)Manα–	Man_3
3-O-su-Galβ-	β-D-galactose-3-sulfate
Neu5Acα–	α-N-acetylneuraminic acid
Neu5Acα2-3Galα1-4GlcNAcα-	3'SLN
Galα1-4Galβ1-4Glcβ-	P_k, Gb_3
Galα1-3GalNAcβ-	Tαβ

Galβ1-3Galβ-	Galβ3Gal
Galβ1-3(Fucα1-4)GlcNAcβ–	Le^a
Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ–	Le^b
Fucα1-2Galβ1-3GlcNAcβ-	Le^d (H type 1)
Galβ1-3GlcNAcβ-	Le^c
Galβ1-4(Fucα1-3)GlcNAcβ–	Le^x
Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ-	Le^y
Galβ1-4Glcβ-	Lac
Galβ1-4GlcNAcβ-	LacNAc
Galβ1-3GalNAcα-	TF
Fucα1-3GlcNAcβ–	Fucα3GlcNAc
Fucα1-4GlcNAcβ–	Fucα4GlcNAc, Le
GalNAcα1-3GalNAcβ-	Fs-2
GalNAcα1-3GalNAcα-	core 5
Galα1-3GalNAcα-	Tαα
Neu5Acα2-3Galβ1-3GlcNAcβ-	3′-SiaLe^c
Galα1-2Galβ-	Galα2Gal
Galβ1-3GalNAcβ-	Tββ
GlcNAcβ1-4GlcNAcβ-	$(GlcNAc)_2$
Neu5Acα2-6GalNAcα–	sTn
Fucα1-2Galβ1-3GalNAca–	H type 3
Neu5Acα2-3Galα1-4Glcα–	3′-SL
Neu5Aca2-3Galβ1-3(Fucα1-4)GlcNAcβ–	sLe^a
Neu5Aca2-3Galb1-4(Fuca1-3)GlcNAcb-	sLe^x
Neu5Acα2-6Galα1-4Glcα–	6′-SL
6-O-su-GlcNAcβ-	β-N-acetyl-D-glucosamine-6-sulfate
O-su-3Galβ1-3(Fucα1-4)GlcNAcβ-	3′-O-su-Le^a
O-su-3Galβ1-4(Fucα1-3)GlcNAcβ-	3′-O-su-Le^x
3′-O-su-LacNAcβ-	3′-su-LacNAc
3′-O-su-Galβ1-3GlcNAcβ-	3′-su-Le^c
Galα1-6Glcβ-	melibiose
Galα1-3Galβ1-4GlcNAcβ-	Galα1-3'LacNAc
GlcNAcα1-3Galβ1-3GalNAcα-	GlcNAcα1-3'TF
Neu5Acα2-8Neu5Acα2	$(Sia)_2$
Neu5Acα2-8Neu5Acα2-8Neu5Acα2	$(Sia)_3$
GlcNAcβ1-3Galβ1-3GalNAcα-	GlcNAcβ1-3'TF
Galβ1-2Galβ-	Gal2βGal
Galβ1-4(6-O-su)GlcNAcβ-	6-O-su-LacNAc
Galβ1-3(GlcNAcβ1-6)GalNAcα-	core 2
Fucα1-2Galβ1-3GalNAcβ-	H type 4
Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-	LNT
Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-	LNnT
Neu5Acα2-3Galβ-	GM4
Neu5Acα2-6Galβ-	Neu5Ac6Gal
GalNAcα1-3(Fucα1-2)Galβ-	A_{tri}
Galα1-3(Fucα1-2)Galβ-	B_{tri}
GalNAcα1-3Galβ-	A_{di}
Galα1-3Galβ-	B_{di}
Fuca1-2Galβ1-4GlcNAcβ-	H type 2

6´-su-LacNAcβ-	6'-O-su-LacNAc
Fucα1-2Galβ-	H_{di}
3´-O-su-Galβ1-3GalNAcα-	3´-O-su-TF
GlcNAcβ1-3Galβ1-4GlcNAcβ	GlcNAcβ1-3'LacNAc
GalNAcβ1-3GalNAcβ-	di-GalNAcβ
Neu5Acα2-3Galβ1-3GalNAcα-	3´-SiaTF
GlcNAcβ1-3GalNAcα-	core 3
GlcNAcβ1-6GalNAcα-	core 6
GlcNAcβ1-3(GlcNAcβ1-6)GalNAcα-	core 4
Neu5Acα2-6(Neu5Acα2-3Galβ1-3)GalNAcα-	3,6-SiaTF
Galβ1-3(NeuAcα2-6)GalNAcα-	6-SiaTF
Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-	YDS
Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-	9-OS
GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-	7-OS
Neu5Acα2-3(Neu5Acα2-6)GalNAcα-	3,6-SiaTn
Neu5Acα2-3GalNAcα	3-SiaTn
Neu5Acα2-6Galβ1-4GlcNAcβ-	6´SLN
Gal	Galactose
Glc	Glucose
Man	Mannose
Neu5Ac	N-Acetylneuraminic acid
Fuc	Fucose
Lac	Lactose
GlcNAc	N-Acetylglucosamine
GalNac	N-Acetylgalactosamine
LacNAc	N-acetyllactosamine

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the following examples are intended to illustrate but not limit the scope of invention described in the claims.

Examples

Example 1

This example describes various exemplary materials and methods.

Cell Culture

Human pancreas carcinoma cell line BXPC-3 and human stomach adeno carcinoma cell line 23132/87 were cultured in RPMI 1640 (PAA, Vienna, Austria) supplemented with 10% heat inactivated FCS (PAA, Vienna, Austria), 2mM L-glutamine and penicillin/ streptomycin (both 1%, PAA). SAM-6 antibody producing hybridoma cells were grown in cell culture flasks

(175cm^2) in AIM/V serum-free medium (Invitrogen, Karlsruhe, Germany). All cells were incubated in a humidified, 7% CO_2 atmosphere at 37°C.

SAM-6 antibody purification

For purification of SAM-6 antibody, cell culture supernatant was collected and purified via cation-exchange column (HiTrap SP FF column, Amersham Bioscience, Freiburg, Germany) using a fast liquid chromatography system (FPLC). After column equilibration with starting buffer (20mM phosphate buffer pH 5.9) cell culture supernatant (same pH) was applied to the column. Unbound proteins were eluted with starting buffer, while bound antibody was displaced by increasing salt concentration with 75% elution buffer (20mM phosphate buffer, 1M NaCl pH 8.0). Antibody containing fractions were diluted in 0.9% sodium chloride, sterile filtered and stored at–70°C until use. Purity of the antibody was determined by SDS gel electrophoresis and activity confirmed by immunhistochemistry and functional assays.

Preparation of Tumor Cell Membrane Extract (*for antigen purification*)

For purification of SAM-6 antigen (SAM-6/R glycoprotein), membrane proteins were isolated from human stomach adeno carcinoma cell line 23132/87 using the ProteoExtract™ Native Membrane Protein Extraction Kit (M-PEK)(Calbiochem, Darmstadt, Germany). The whole procedure of the native 2-step extraction of integral and membrane-associated proteins was performed at 4°C or on ice. All required buffer solutions and reagents were provided in the M-PEK.

3-5 x 10^6 tumor cells (frozen cell pellet) were washed twice with 2ml ice cold Wash buffer. After 10min centrifugation at 300 x g and 4°C, supernatant was removed without disturbing the pellet and discarded. The pellet was carefully resuspended in 2ml ice cold extraction buffer I containing 10µl protease inhibitor cocktail. After 10min incubation at 4°C under gentle agitation on a rotary shaker, insoluble material was sedimented by centrifugation at 16,000 x g and 4°C for 15min. For the purpose of control, an aliquot of the supernatant, enriched in soluble proteins, was transferred into a sample tube without disturbing the cell layer. The rest of the fraction was discarded. In a second extraction step, 1ml extraction buffer II containing 5µl protein inhibitor cocktail, was added to the pellet of step I, carefully mixed by using a pipette and incubated for 30min at 4°C under gentle agitation. Insoluble material was centrifuged for 15min at 16,000 x g and 4°C. The supernatant, now enriched in integral membranes and membrane associated proteins, was completely transferred into a new sample tube without disturbing the debris pellet. Aliquots were stored at -20°C after determination of the protein content by BCA method using bovine serum albumin as standard.

Western Blot Analysis

Reducing SDS-Page gels (8%) and Western blotting of membrane proteins were performed using standard protocols. In short, blotted 0.2µm nitrocellulose membranes were blocked with PBS containing 0.05% (v/v) Tween-20 and 5% (w/v) low fat milk powder, followed by incubation for 1 h with 20µg/ml SAM-6 human IgM antibody or unrelated human control IgM (Chrompure IgM, Dianova, Hamburg, Germany). The secondary antibody (peroxidase-coupled rabbit anti-human IgM antibody 1:1,000; Dianova) was detected with the SuperSignal chemiluminescence kit from Pierce (Perbio Science Deutschland GmbH, Bonn, Germany).

Purification of SAM-6 Receptor

Membrane fraction obtained from tumor cell line 23132/87 after extraction with M-PEK was used to purify the antigen of SAM-6 antibody. For the first of step of purification size exclusion chromatography was used. 10ml M-PEK extract (1mg/ml) was injected to a Superdex 200 column (XK16/60; Amersham Biosciences, Uppsala, Sweden) at a flow rate of 1ml/min using a super loop system and a fast protein liquid chromatography unit (FPLC) (Amersham Pharmacia Biotech, Freiburg, Germany). The column was previously equilibrated with buffer A (100mM Tris/HCl pH 7.5, 40mM NaCl, 2mM EDTA, 1% TritonX-100) and eluted with the same buffer at a flow rate of 2ml/min. Fractions of 2ml each were collected and fractions corresponding to the peak of SAM-6 receptor activity, were combined and applied to an equilibrated anion-exchange column (HiTrap™ Sepharose Q XL, 5ml; Amersham Biosciences, Uppsala, Sweden). Unbound components were eluted with buffer A, whereas bound protein were released by increasing salt concentration via linear step gradient using buffer B (100mM Tris/HCl pH 7.5, 1M NaCl, 2mM EDTA, 1% TritonX-100).

Again a flow rate of 2ml/min was used and fractions of 2ml were collected and monitored at 280nm. After concentrating the eluate via acetone precipitation at -20°C over night, protein pellet was dissolved in 1x SDS buffer and examined by SDS-Page and Western Blot analysis for reaction with SAM-6 antibody. Positive bands were excised from Coomassie stained gel and sequenced.

Protein Identification by Peptide Mass Mapping

Protein Sequencing was performed by TopLab (Martinsried, Germany). After one-dimensional SDS-Page with Coomassie staining a protein band having an estimated molecular mass of 80kDa was excised and after reduction and alkylation with iodoacetamide, the band was in-gel digested with trypsin. The pool of tryptic peptides was desalted via ZipTipC18 and analyzed by Matrix assisted Laser Desorption Ionization (MALDI) Mass Spectrometry (Voyager-DE STR; Applied Biosystems, CA, USA) followed by databank research (Profound and Mascot versus NCBI). Hits

for the best matching protein candidates (probability of 1.00) were compared using the Basic Local Alignment Search Tool.

Transfection studies with small interfering RNA of Grp78

Cell line BXPC-3 was used for SAM-6 receptor binding studies after transfection with small interfering RNA (siRNA). SiGENOME SMARTpool reagent against human HSPA5, pre-designed and validated siRNA for silencing the target molecule Grp78, was purchased from Dharmacon (Lafayette, CO, USA). Silencer™ Negative control, as control for non-specific effects on gene expression caused by siRNA introduction, was purchased from Ambion (Cambridge, UK).

As further controls served mock transfected (without siRNA) and untreated cells (grown in complete RPMI-1640, not transfected). For transfection of siRNA siLentFect™ Lipid (BioRad Labratories, CA, USA) was used. Additionally, to control transfection of siRNA into the cells, Silencer CY3 GAPDH siRNA (Ambion, Cambridge, UK) was transfected and verified using confocal microscopy.

The day before transfection 24-well plates were seeded with 1 x 10^4 cells in 1ml complete growth medium per well (50% confluent the following day). Plates were incubated over night at 37°C and 7% CO_2 30 min prior transfection, the medium was carefully replaced by 0.4ml fresh and complete medium per well. For each well 100µl transfection mixture was prepared and added to the cells. The mixture consisted of 49µl serum-free OptiMEM (Invitrogen, Karlsruhe, Germany), 1µl siLentFect™ Lipid combined with 50µl siRNA after incubation for 20 min at room temperature. Final concentration of SiGENOME SMARTpool was 100nM and 25nM of control siRNAs. Transfected cells were incubated over a period of 24, 48 and 72 h at 37°C and 7% CO_2 atmosphere. Knockdown of Grp78 after the indicated time was monitored by FACS analysis.

Detection of Grp78 protein knockdown by FACS analysis

Tumor cell line BXPC-3 was transfected with siGENOME siRNA against human GRP78. Cells were harvested after 1 and 3 days by gentle detaching with Trypsin/ EDTA (PAA, Vienna, Austria). After 1 and 3 days protein levels of GRP78 were monitored by FACS analysis. Respectively 2 x 10^5 cells were incubated on ice with SAM-6 antibody (100 µg/ml), anti-GRP78 antibody (100 µg/ml; clone ET-21, Sigma, Taufkirchen, Germany) or anti-CD55 antibody (1:50; clone 143-30, DPC Biermann, Bad Naunheim, Germany) for 30 minutes. Unrelated human IgM (Chrompure IgM, Dianova, Hamburg, Germany) and rabbit/ mouse IgG (Sigma, Taufkirchen, Germany) served as negative controls. Incubation with FITC-labeled secondary antibodies (rabbit antihuman IgM antibody, Dako, Hamburg, Germany; goat anti-rabbit IgG or goat anti-mouse

IgG, both Acris, Hiddenhausen, Germany) for 15 minutes followed. Cells were analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, California) using WinMDI software.

Apoptosis Assay with SAM-6 on Transfected Tumor Cells

The extent of antibody-induced apoptosis on BXPC-3 cells before and after transfection with GRP78 siRNA was detected by the Cell Death Detection ELISAPLUS (Roche, Mannheim, Germany) according to the manufacturer's protocol. 48 hours after transfection 1 x 10^4 cells were plated on 96-well plates and incubated in presence of 100 µg/ml SAM-6 antibody or unrelated IgM control (Chrompure human IgM, Dianova, Hamburg, Germany) for 4 h at 37 °C and 7% CO_2 in a humidified atmosphere. To demonstrate normal growth, cells were supplemented with complete growth medium.

Preparation of Tumor Cell Membrane Extracts *(for glycosidase assay)*

Cell line BXPC-3 was grown to 80% confluency on 100mm cell culture plates. Culture plates were washed twice with phosphate buffered saline pH 7.4 (PBS), adherent cells were harvested in PBS followed by centrifugation for 5 min at 1,500rpm. Cells were resuspended in ice cold hypotonic buffer (20mM HEPES, 3mM KCl, 3mM $MgCl_2$) and incubated for 15min, followed by 5 min ultrasonic vibration. Nuclei and cytoskeletons were pelleted by 10min of centrifugation at 10,000 x g. The supernatant was centrifuged for 30min at 100,000 x g in a SW28 rotor to yield the microsomal pellet, and finally carefully resuspended in modified lysis buffer (50mM Tris-HCl, pH 7.4; 150mM NaCl; 1mM EDTA; 1% Nonidet NP-40 and 0.25% sodium deoxycholate). Insoluble material was removed by centrifugation for 10min at 16,000 x g. The supernatant was transferred into a new sample tube without disturbing the debris pellet. Aliquots were stored at -20°C after determination of the protein content by BCA method using bovine serum albumin as standard. The whole procedure was performed at 4°C or on ice. Complete protease inhibitor tablets (Roche Biochemicals, Mannheim) were added to all solutions.

Glycosidase Assay

Membrane extracts of BXPC-3 cells, prepared by differential centrifugation, were used for glycosylation studies. To cleave all types of N- and O-linked carbohydrate chains, the membrane extract was denaturated in buffer containing 1% sodium dodecylsulfate and 1% ß-mercaptoethanol for 3 min at 95°C. The denaturated extract was diluted with reaction buffer (PBS pH 7.4, 1% nonidet NP-40, 1% ß-mercaptoethanol) to the final protein concentration of 0.5 mg/ml. For deglycosylation of O- and N-linked carbohydrates, aliquots of 100µl were incubated either with 10U N-glycosidase F (Roche Applied Science, Mannheim, Germany) or 5mU O-glycosidase (Roche Applied Science, Mannheim, Germany) at 37°C over night. Untreated extract

in reaction buffer served as control. The extent of deglycosylation was analyzed by SDS-Page and Western Blotting procedure.

Glycosidase Assay on cytospins

$4x10^5$ human pancreas carcinoma cells (BXPC-3) were washed and resuspended in 1ml Dulbecco's phosphate buffered saline pH 7.2 (Sigma, Taufkirchen, Germany). Incubation for 4 hours at 37°C with 5U/ml N-glycosidase or 20mU/ml O-glycosidase (both Roche Applied Science, Mannheim, Germany) followed. Untreated cells in phosphate buffer served as control. Corresponding aliquots of 100µl were washed and spun on coverslips at 500rpm for 2 min. Dried cytospins were fixed with acetone and stained immunohistochemically.

Immunohistochemical staining of cytospins

Dryed cytospins were fixed with acetone (10 minutes) and blocked for 30min with low fat milk powder/ PBS (4%). After washing with Tris/NaCl , the coverslips were incubated with antibody SAM-6 (50µg/ml) or control antibodies for 30 minutes. As negative control served unrelated human IgM (Chrompure IgM, Dianova, Hamburg, Germany) in the same concentration and as positive control anti-CD55 antibody (1:30; clone 143-30, DPC Biermann, Bad Naunheim, Germany). After washing with Tris/NaCl incubation with secondary antibodies for 30 minutes followed (peroxidase-labeled rabbit antihuman or rabbit antimouse conjugate 1:50). After final washing with Tris/NaCl and incubation in PBS for 10 minutes, staining was performed with diaminobenzidine (0.05%)-hydrogen peroxide (0.02%) for 10 min at room temperature. The reaction was stopped under running tap water and the coverslips counterstained with hematoxylin. After mounting with glycerol-gelatin, the extent of deglycosylation was visual analyzed using light microscopy.

Labeling of SAM-6 antibody with fluorescein isothiocyanate

Endocytosis of SAM-6 antibody was determined on human pancreas carcinoma cell line BXPC-3. For immunofluorescence studies conjugation of monoclonal antibody SAM-6 and isotype control IgM (Chrompure IgM, Dianova, Hamburg, Germany) was performed with Fluoro Tag FITC Conjugation Kit (Sigma-Aldrich, Saint Louis, USA) according manufacturer's protocol. Purified antibody SAM-6 or IgM isotype control were dissolved in sodium carbonate-bicarbonate buffer pH 9.0 at a concentration of 2.5mg/ml. Therefore, consisting buffer was exchanged with sodium carbonate-bicarbonate buffer pH 9.0 over a Sephadex™ G-25 column. The final concentration of antibodies was approx. 1.7 mg/ml (dilution factor 1.5). A solution of fluorescein isothiocyanate (FITC) in 2ml 0.1M carbonate-bicarbonate buffer (per FITC vial) was prepared just prior to addition to antibodies. 50µl of the FITC solution was drop-wise added to 0.2ml of

each antibody solution and incubated for 2 h at room temperature in the dark with gentle stirring. Finally, a molar ratio of 20:1 of FITC (MW 389) to IgM (MW 900) was used in the reaction mixture, expected fluorescein-antibody conjugates with F/P ratio from 3 to 6. Cells were trypsinized and left on ice for 1h.

FITC labeled antibodies were separated from free FITC by gel filtration on a Sephadex™ G-25 column. After column equilibration with at least 30ml PBS the reaction mixture was applied to the top of the column gel bed. Column elution started with 10ml PBS, collecting 0.25ml fractions. During elution, the first of two appearing peaks contained the conjugate. Protein content was determined by BCA method using bovine serum albumin (Roth, Karlsruhe, Germany) as standard.

SAM-6 Endocytosis

Endocytosis was determined for SAM-6 antibody on human pancreas carcinoma cell-line BXPC-3. Monoclonal antibodies SAM-6 (purified) and isotype control (ChromPure IgM, Dianova, Hamburg, Germany), were conjugated with Fluoro Tag FITC Conjuagtion Kit (Sigma-Aldrich, Saint Louis, USA) as described above. Conjugated antibodies at a final concentration of 40µg/ml were directly given to 1 x 10^6 cells and incubated for 30, 60, 120 minutes at 37°C. Cells were harvested, rinsed and resuspended in phosphate buffered saline pH 7.4 (PBS). 100µl of each cell suspension was fixed on slides. Finally the slides were mounted with Fluorescent Mounting Medium (DakoCytomation, Carpinteria, USA) and analyzed by confocal microscopy.

Lipid staining with Sudan III

For intracellular lipid staining pancreas carcinoma cells BXPC-3 were grown on glass slides. Adherent cells were incubated for 48h with 100µg/ml SAM-6 antibody, anti-Grp78 (cloneET-21, Sigma, Taufkirchen, Germany) or unrelated control (Chrompure IgM, Dianova, Hamburg, Germany). After two washing steps with PBS, cells were fixed for 5 minutes with 60% isopropanol. Before use, a 60% solution of a Sudan III stock (0.5% Sudan III in 100% isopropanol) was matured over night, filtered and added to the fixed cells. After 40 minutes cells were washed with distilled water, differentiated in 60% isopropanol, washed again and counterstained for 6 minutes with hematoxylin. Finally cells were rinsed with water for 10 minutes and mounted with glycerol gelatine. The extent of lipid inclusions was visualized using light microscopy.

Cytochrome C Assay

For screening whether cytochrome c was released during apoptosis induced by SAM-6 the Cytochrome C ELISA Kit (Calbiochem, LaJolla, USA) was applied. In short, 1.5 x 10^6 stomach carcinoma cells (23132/87) were incubated with 200 µg/ml purified SAM-6 or unrelated IgM

antibody for one and four hours respectively. After trypsinization the cells were washed three times with cold PBS, resuspended in lysis buffer and incubated for one hour at RT with gentle mixing. After three washing steps with ice-cold PBS and centrifugation at 1,000 x g for 15 min, the supernatants were transferred into a fresh tube and diluted (1:10) with Calibrator Diluent RD5P (1x). Then a mixture (1:1) of each of the diluted samples with Calibrator Diluent RD5P (1x) was add into the micro-titer plate delivered in the kit. A incubation for two hours at RT and then a washing with 0.4 % washing buffer followed. Then the Cytochrome C Conjugate was put into each well. The incubation and washing steps were repeated in the way before. After the addition of Substrate Solution (1:1 mixture of Color Reagent A and B) to each well the plate was incubated for 30 min at RT. The measurement at 415 nm (reference wavelength 540 nm) was carried out after the addition of Stop Solution.

Caspase Assay

For screening antibody-treated cells for Caspase-2, -3, -6, -8, and -9 activity the Apo Target™ Colorimetric Protease Assay Sampler Kit (Calbiochem, LaJolla, USA) was used following the suppliers manual. In short, $3x10^6$ stomach carcinoma cells (23132/87) were incubated with 200 µg/ml purified SAM-6 or unrelated IgM antibody for one and four hours respectively. After trypsinization, the cells were resuspended in cold lysis buffer. They were incubated for 10 min on ice and centrifuged for 1 min at 10,000 x g. To determine the amount of protein in the cell lysates Bradford assay was applied. Each cytosol extract was diluted to a protein concentration of 4 µg/ml. Then reaction buffer containing DTT and the various conjugated protease substrates were added to the samples in a 96-well micro-titer plate. A mixture (1:1) of lysis and reaction buffer served as a blank. After incubation for two hours at 37 °C and 7% CO_2 in a humidified CO_2 incubator the absorption and thus the extent of caspase activity was measured in an ELISA reader at 415 nm. For experiments with caspase-3-inhibitor the Caspase-3 Cellular Activity Assay Kit (Calbiochem, LaJolla, USA) was used following the suppliers manual, using similar conditions as described above.

***In vivo* studies**

To determine the effects of SAM-6 antibody on tumor cell growth *in vivo*, a nude mouse/human stomach carcinoma cell system was used. Briefly, 2 x 10^6 stomach carcinoma cells (23132/87) were injected subcutaneously (s.c.) into 6-7 week old NMRI-nu/nu mice (Harlan Winkelmann GmbH, Borchen, Germany) followed by injections of antibody SAM-6 antibody when tumors reached visible size. 50, 250, 500 or 750 µg antibody was given respectively at days 9, 11, 14, 16 and 17 i.p. post carcinoma cell injection. Control mice were injected with 750 µg unrelated human IgM (Chrompure IgM, Dianova, Hamburg, Germany). Visible tumor growth was

measured macroscopically during the experiment. The study was terminated when tumors had reached maximal tolerable size (day 18), whereupon the mice were sacrificed, tumor volume respectively tumor weight was determined, and organs and tissues inspected for the spread of tumors and other alterations.

ELISA for SAM-6 scFv Binding to LDL

Materials: The plate washer (BIORAD 1575 Washer). MAXISORP 96 well immunoplates from Nunc. SecureSeal™ Thermal Adhesive Sealing Film from Eppendorf. The plate reader (BIORAD 680 reader). Pipettes (10-100µl) from Eppendorf

Coating antigen to microplate: Dilute the antigen to a final concentration of 10µg/ml in 1 x PBS pH 6.5 buffer (e.g. 200µl of the 100µg/ml LDL in 1.8ml 1 X PBS pH6.5). Coat 96 wells of a MAXISORP Nunc immunoplate by pipeting 50µl of the diluted antigen onto each well (0.5µg/well) Test samples containing pure antigen are usually pipeted onto the plate at less than 2µg/ml. Pure solutions are not essential, but as a guideline, over 3% of the protein in the test sample should be the target protein. (antigen). Antigen protein concentration should not be greater than 20µg/ml as this will saturate most of the available sites on the microtitre plate. Ensure the samples contain the antigen at a concentration that is within the detection range of the antibody. Next, cover the plate with an adhesive plastic and incubate for 2h at room temperature, or 40C overnight. The coating incubation time may require some optimization. Then, remove the coating solution and wash the plate 3 times by filling the wells with 200µl 1 x PBS pH6.5, 3 times with 1 x PBS-T pH6.5 and 3 times with 1 x PBS pH6.5. The solutions or washes are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel Or Remove the solution by flicking the plate over a sink. Wash the plate 3 times with 1 x PBS pH6.5, 3 times with 1 x PBS-T pH6.5 and 3 times with 1 x PBS pH6.5 using the plate washer. The remaining drops are removed by patting the plate on a paper towel.

Blocking: Block the remaining protein-binding sites in the coated wells by adding 250µl blocking buffer, 10% skim milk in 1 x PBS pH6.5, per well. Cover the plate with an adhesive plastic and incubate for 1h at room temperature or if more convenient overnight at 40C. Wash the plate.

Confocal Microscope Studies of Binding to Cancer Cells

Place a single cover slip on each well in a 12 well plate. Grow the cells on cover slip plated at a density of 2 x 10^4 cells/cover slip (500-1000µl of cell culture/well). Incubate the plate in 5% CO_2 in air at 37°C, and then allow cells to grow overnight (70-80% confluent).

To fix cells, wash cells once in ~500µl 1 x PBS pH 7.5, fix cells in 300-400µl 4% PFA 15min, wash twice in 1 x PBS pH7.5, quench free aldehydes in ~500µl 50mM NH4Cl/PBS 10min, and then wash twice in 1 x PBS pH 7.5. Leave in 1 x PBS pH 7.5 for 5 min. Plate with the cover slips

can be stored at 4^0C in 1 x PBS pH 7.5 with 0.02% Na azide for few weeks. If necessary permeabilize cells in ~500µl of 0.1% TX-100 4min only, wash 3 x in 1 x PBS pH 7.5, and then block in ~500µl of 15% FCS/1 x PBS pH7.5 for 20 min.

To stain cells, wash once in 1 x PBS pH 7.5 and then add ~300-400µl dilute primary Ab (diluted in 5% FCS/1 x PBS high salt pH6.5, 1:100 or neat) per cover slip and incubate at RT 45 min-1h. Afterwards, wash 6 x in 1 x PBS pH 7.5 over 30 min, dilute secondary conjugate (1:100 for FITC and 1:500 for Alexa fluor 488) in 5% FCS/1 x PBS pH 7.5 and clarify at ~14,000g in Microcentrifuge at 4°c for 10min. Add ~300-400µl secondary conjugate to each cover slip and incubate at RT for 30 min-45 min (cover the plate with aluminium foil). Wash 6 x in 1 x PBS pH 7.5 over 30 min. Stain nuclei with DAPI 1/10,000 (of 5mg/ml stock) for 5min ~300µl (Vortex the tube before use). Wash 3 x in 1 x PBS pH 7.5, rinse in H_2O (to remove salt) and blot dry cover slip (from edge without touching surface with cells) and mount in 10µl of mounting fluid (thawed at RT) on clean microscope slides. Store in fridge with aluminium foil until ready to view

Example 2

This example includes a description of purification and analysis of SAM-6/R glycoprotein.

Western Blot Analysis on Membrane Extracts

The isolation and characterization of SAM-6 antigen was performed on tumor cell line 23132/87 and BXPC-3. SAM-6 antibody bound to an antigen with a relative molecular mass of about 80kDa (Fig. 3). On both cell lines, Western Blot showed comparable results in the pattern of appearing bands. Also, there was no significant difference, whether we used differential centrifugation or the MPEK method to prepare the samples. Nevertheless, for purification of the antigen we employed the method of native Membrane Extraction Kit, because of the greater enrichment and fewer unspecific bands at the lower molecular weight. To find out unspecific binding of IgM antibodies, unrelated human IgM was used for control. Non specific binding could be observed at about 50kDa. Additional prominent protein bands at a molecular mass of about 60 and 100kDa shaped up as impurities from membrane fractionation during preparation.

Purification and Identification of Human SAM-6 Receptor by Peptide Mass Mapping

To identify the 80-kDa protein binding to SAM-6 antibody on Western Blot, two steps of column chromatography was used to purify and MALDI mass spectroscopy to identify the protein. Figure 4 shows a representative chromatogram after the first step (size exclusion chromatography). Fractions, containing the protein binding to SAM-6 antibody, were analyzed by SDS-PAGE, followed by Western Blot and Coomassie staining. Positive fractions are branded in Figures 5 and

6. The second step followed using anion exchange chromatography (chromatogram see Fig.7). Subsequent analysis by SDS-PAGE is shown in Figures 8 and 9. Arrows indicate the band representing the 80kDa protein, that finally was excised and sequenced. Protein sequencing was performed by TopLab (Martinsried, Germany).

A sequence database search with the matched set of tryptic peptide masses calculated human Grp78 (accession no. NP_005338.1) as the highest ranking candidate. A total of 21 tryptic peptide masses were assigned to Grp78 protein corresponding to an amino acid sequence coverage of minimum 35% (Figs. 10 and 11). The peptide mass error was less than 50ppm. Because of the high number of assigned peptides and the high sequence coverage, Grp78 protein was identified from the database. Alignment of experimentally derived peptide sequences and the protein database sequence of human Grp78/BiP is shown in Figure 11. Bold text are sequences that display the masses obtained by MALDI peptide-mass fingerprinting and are marked by stars in the peptide mass map (Fig. 10).

Structure analysis

Structure analysis for potential trans-membrane region and glycosylation sites was performed using sequence analysis tools of the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (www.expasy.org). Beside signal regions at amino-acids 1 to 17 and 650 to 654, Grp78 shows a possible transmembrane region from amino-acid 220 to 237 and potential extra-cellular O-glycosylation sites at amino-acid residues166 and 184. N-glycosylation sites could not be determined (Fig. 11).

Example 3

This example includes a description of Grp78 expression inhibition studies with siRNA.

Knockdown of Grp78

Studies were performed to investigate if silencing of Grp78 gene, coupled with knockdown of Grp78 reduced binding of SAM-6 antibody on BXPC-3 tumor cells.

Transfection of tumor cell line BXPC-3 with Grp78 siRNA reduced protein expression and binding of SAM-6 antibody. On cells transfected with Negative silencer™ siRNA (unrelated siRNA) strong binding of SAM-6 and control antibodies is detectable during the whole incubation time, showing no significant effects of Grp78 expression. Cells transfected with Grp78 siRNA show reduced binding of anti- Grp78 (76% reduction) and SAM-6 (70% reduction). Silencing did not affect the expression of other cell surface membrane molecules. Binding of anti-CD55 after transfection was as strong as before (Figs. 12 and 13). Knockdown of surface

located Grp78 reduces target protein expression and binding of SAM-6 antibody (Figs. 12 and 13).

Apoptosis Assay

Apoptotic activity was measured by Cell Death Detection ELISA^{PLUS} (Roche) to demonstrate the extent of antibody-induced apoptosis of transfected tumor cells.

Transfected cells were incubated with 100 µg/ml SAM-6 antibody for 4h. Unrelated human IgM in the same concentration was negative control. Tumor cells transfected with siRNA against human Grp78 show a clear reduction of the apoptotic activity induced by SAM-6 antibody compared to untreated cells and cells transfected with unrelated siRNA (Fig. 14). Calculation of apoptotic cell content was related to the content of cells grown in complete medium.

Example 4

This example includes a description of studies on the effects of glycosidase treatment of SAM-6/R glycoprotein on SAM-6 antibody binding.

Glycosidase Assay

To confirm binding of SAM-6 antibody on O-linked carbohydrates, the effect of deglycosylation of membrane extracts of tumor cell line BXPC-3 was studied. Membrane extracts were deglycosylated under reduced conditions with O- and N- glycosidase over night, afterwards separated via SDS-Page and analyzed by western blotting. After incubation with glycosidases the molecular weight of the prominent 80kDa protein clearly decreases and so does the binding activity of SAM-6 (Fig. 15).

Glycosidase Assay on cytospins

To ascertain SAM-6 antibody binding on an O-linked carbohydrate moiety on the tumor cell surface a Glycosidase Assay was done on cytospins. Human pancreas carcinoma cells (BXPC-3) were incubated with N-glycosidase or O-glycosidase. After preparation of cytospins, a staining was performed with SAM-6 antibody and anti-CD55.

Immunohistochemical staining with SAM-6 antibody shows a significant reduction of surface binding when treated with O-Glycosidase. Treatment with N-Glycosidase had no detectable effect on SAM-6 antibody binding (Fig. 16). The cell surface molecule CD55 served as a control for membrane integrity and showed no changed binding after treatment with glycosidases.

Although the data indciate that SAM-6 binding to O-glycosidase treated cells was significantly reduced, suggesting possible involvement of a carbohydrate moiety in the eptiope to which SAM-6 antibody binds, subsequent data described in Examples 12 and 13 indicate that SAM-6 binds to

a cell free wheat germ translated grp78 and deglycosylated LDL meaning that carbohydrates do not appear necessary for SAM-6 binding to antigen.

Example 5

This example includes a description of studies of SAM-6 antibody binding to SAM-6/R glycoprotein present on cells.

SAM-6 Endocytosis

SAM-6 antibody binds to cell membrane SAM-6/R glycoprotein. This antibody/receptor binding initiates the accumulation of lipids and the apoptotic cascade. SAM-6 antibody also binds to oxLDL and carries this into cancer cells. Lipoproteins are normally internalized by a receptor-mediated endocytosis.

To study what happens after SAM-6 antibody binds to a cancer cell membrane, SAM-6 antibody and an isotype control were conjugated with FITC. Conjugated antibodies in the presence of LDL were directly given to human pancreas carcinoma cell-line BXPC-3 and incubated for 30, 60, 120 minutes. Cells were finally analyzed by confocal microscopy. After 30 min of incubation with SAM-6 antibody, binding to the cell surface could be observed (Fig.17A). After 60 min SAM-6 antibody is concentrated at the membrane, seen as a typical formation of "capping" (Fig. 17B). One hour later SAM-6 antibody is completely internalized into the cell (Fig. 17C). In comparison, the labeled control antibody did not show similar events (Fig. 17D, E, F). It can be assumed that oxLDL is carried into the cell together with the antibody.

Lipid staining with Sudan III

To examine SAM-6 antibody-induced intracellular lipid accumulation, we performed a staining with Sudan III. This dye is specific for the detection of neutral lipids and fatty acids. Fig. 18 shows the data obtained after 48h of incubation with SAM-6 antibody, anti-Grp78 or unrelated human control IgM on pancreas carcinoma cell-line BXPC-3. When treated with SAM-6 antibody, the tumor cells show clearly an antibody-induced accumulation of neutral lipid droplets (Fig. 18). In contrast, cells treated with anti-Grp78 and unrelated control antibody respectively show no significant accumulation of lipids. These data indicate that the intracellular accumulation of lipids is a direct effect mediated by SAM-6 antibody.

SAM-6 apoptosis

SAM-6 induced apoptosis may be a consequence of the disturbed lipid homeostasis. So far, however, nothing was known about the pathway between antibody-binding, lipid accumulation and the ultimate cell death nor whether caspases are activated. A better understanding of the signaling pathway activated by SAM-6 will make a further contribution to innovative therapies

for the fight against cancer. Caspases were therefore studied for activation during the SAM-6 induced apoptotic process.

To examine whether Cytochrome C was set free in gastric cancer cells after incubation with SAM-6 antibody and control IgM respectively, the Cytochrome C ELISA Kit was applied. The sandwich enzyme immunoassay technique indicated that Cytochrome C was released at a higher level in cells treated with SAM-6 antibody than in the cells treated with the unrelated, human IgM after 1h. Additionally, the amount of the polypeptide in both samples differed more than after four hours of incubation. Also observable is a decrease in the SAM-6 sample after four hours (Fig. 19). This clearly indicates that in the SAM-6 induced pathway a perturbation of the mitochondria occurred leading to the break of the outer membrane and resulting in the Cytochrome C release. The Cytochrome C level in SAM-6 antibody treated cells came close to that of control after four hours of incubation signifying that the mitochondrial breakdown only occurred at an early stage of the pathway.

In order to determine whether caspases and which caspases are induced by SAM-6 antibody, the Apo Target™ Colorimetric Protease Assay Sampler Kit was used. After 1h incubation with the antibodies the activity of caspase-2, -3, -6, -8, and -9 was measured. The initiator caspases -8 and -9 but not -2 were activated in cells treated with SAM-6 antibody compared with those treated with the unrelated human IgM control already after 1h (Fig. 20A). In addition, after 4hr an activation of the effector caspases -3 and -6 could be detected.

To exclude artifacts, a study using a caspase-3 inhibitor was prepared additionally as an example. Figure 20B shows the suppressed caspase-3 activity when the inhibitor was added. In absence of the caspase-3 inhibitor a clear activation of caspase-3 could be observed in the SAM-6 treated tumor cells.

Example 6

This example includes a description of *in vitro* studies of malignant melanoma.

To determine the effect of SAM-6 antibody on melanoma cells, sensitivity of malignant melanoma cells HTB-69 and CRL 1424 to SAM-6 antibody was determined. In brief, cells were trypsinized and diluted with RPMI containing 2%FCS to a concentration of $2x10^5$cells/ml. A 50µl cell-suspension per well were plated onto a 96-well-plate. Antibody was diluted according to 100µg/ml with RPMI (leading to a final concentration of 1% FCS per well). IgM, RPMI and buffer were used as controls. Cells were incubated in an incubator at 37°C for 2, 5, 24 or 48 hours, supernatant was discarded, 30µl Trypsin added per well and incubated for a few minutes, and cells displaced from the bottom of the well observed with a microscope. The reaction was

terminated with 6µl FCS per well, 324µl Guava ViaCount Reagent (Guava Technologies, Hayward, USA) was added per well and mixed gently and incubated for 5min. at room temperature, and the data analyzed with Guava PCA-96.

The data indicate that SAM-6 antibody was able to kill both melanoma cell types. Cell killing was greatest for HTB-69 cells incubated for 48 hours.

Example 7

This example includes a description of *in vivo* studies.

To determine the effects of SAM-6 antibody on tumor cell growth *in vivo*, a nude mouse-human stomach carcinoma cell system was used. A concentration of 2×10^6 cells derived from the human stomach carcinoma cell line 23132/87 were injected intraperitoneal (i.p.) into NMRI nu/nu mice. Nine days after the inoculation of tumor cells, different doses of SAM-6 antibody were injected i.p. Unrelated human control IgM as well as NaCl solution (0.9%) served as negative controls. The antibody was given again on days 11, 14, 16 and 17 post carcinoma cell implantation. Control mice were injected with 750 µg unrelated human IgM (Chrompure IgM, Dianova, Hamburg, Germany).

Throughout the study, tumor growth was controlled macroscopically. After 18 days the mice were sacrificed, tumor volumes were determined and Student's t test was used to compare tumor sizes between treatment and control groups. The tumors which developed during the course of the study showed a significant reduction in volume when treated with SAM-6 antibody (Fig. 21). Moreover, the reduction of tumor volume in mice treated with SAM-6 antibody is dose dependent. Already mice treated with 50µg SAM-6 antibody show a clearly reduced tumor volume compared with the control groups. Animals treated with 250µg respectively 500µg SAM-6 antibody had statistical significantly smaller tumor volumes (t-Test, $p < 0,05$ for both concentrations) when compared with the IgM control. However animals treated with 750µg SAM-6 antibody show no significant reduction in tumor volume.

Example 8

This example includes a description of hybridoma produced SAM-6 antibody heavy and light chain variable region sequencing.

Samples of SAM-6 antibody derived from the hybridoma (6 ug) were elctrophoresed under reducing conditions in a 4-12% Bis-tris gel (Invitrogen) and stained with Commassie Blue R-250. Bands corresponding to the heavy and light chains of the antibody were excised from the gels,

destained and digested with 100 ng of trypsin per sample. After concentration by centrifugal evaporation, the extracts were made up to 10 ul with 1% formic acid.

In-solution tryptic digests were performed as follows. SAM-6 (2 ug in 2 ul) was diluted to 10 ul with10M urea and reduced by the addition of DTT (dithiothreitol) to a concentration of 0.5mM. After 10 minutes at 20C, iodoacetamide was added to 2.5mM and incubated for 4 minutes at 20C. The sample was diluted to a volume of 80 ul containing 50mM ammonium bicarbonate and 100 ng trypsin. Digestion proceeded for 7 hours at 20C.

MALDI Mass spectrometry (MS and tandem MS (MS/MS)

Liquid chromatography /fraction collection: Five ul of digested heavy chain or light chain of SAM-6 antibody was separated using an Agilent 1100 series, HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a cooled autosampler, an eluant degasser and a capillary pump. The system was controlled by Hystar software, version 3.2 (Bruker Daltonics, Bremen, Germany), with Solvent A being water with 0.1% TFA (triflouroacetic acid) and solvent B 100% ACN (acetonitrile) with 0.1%TFA. The sample was injected onto an in-line column (180um x 15um x 3um, 100Angstroms, Acclaim PepMap C18, LC Packings, Dionex, - Amsterdam, Netherlands) and equilibrated with 5% ACN/0.1% TFA at 1uL/min. The samples were resolved with an ACN gradient (5%-17% in 2 minutes, 17%-45%, 36 minutes). The LC system was interfaced directly with the Proteineerfc spotting robot (Bruker Daltonics, Bremen, Germany). Starting 8 minutes after injection, the liquid chromatrography (LC) eluated was deposited onto a 600 or 800 um AnchorChipTM target in 15 second fractions. Simultaneous deposition of 0.5ul 0.1% TFA was also performed to minimise peak tailing. Once all spots were dry, 0.8 (for 600 um) or 1 ul (for 800um) 0.5 ug/ul alpha-cyano-4-hydrozcinnamic acid was deposited onto the raster spots according to the method of Zhang et al (Proteomics 7:2340-2349 (2007)), without washing using 0.5% TFA. Peptide designated calibration spots prior to the addition of matrix. The target was then allowed to air-dry before insertion into the mass spectrometer.

Data acquisition – MALDI Mass spectrometry (MS)

MALDI TOF (time-of-flight) mass spectra were acquired using a Bruker ultraflex III MALDI TOF/TOF mass spectrometer (Bruker Daltonics) operating in reflectron mode under the control ofthe flexControl software (Version 3.0 Bruker Daltonics). The MALDI target geometry, order of spot acquisition, external calibration and data acquisition methods were defined in WARP-LC software (version 1.1 Bruker Daltonics). At each sample position, mass spectra were obtained

from 100-shot increments and summed up to 400 shots using a hexagon raster movement. The laser repetition rate was 200Hz and the laser power remained unchaged through the experiment. The mass accuracy of the intraument using external calibration spots were typically <50 ppm. MS spectra were subjected to smoothing, background substration and peak detection using flexAnalysis (Version 3.0, Bruker Daltonics). The peptide ions detected in each fraction were visualized using WARP-LC Survey Viewer (Bruker Daltonics). Additionally , a complete list of parent precursors that surpassed selection criteria was generated using the program WARP-LC.

Data acquisition – MALDI Tandem mass spectrometry (MS/MS)

MS/MS was performed using laser-induced dissociation (LID). Data acquisition was performed in LIFT mode in a fully automated fashion using flexControl and WARP-LC software. MS/MS spectra obtained were subsequently subjected to smoothing, background subtraction and peak detection using flexAnalysis.

Data analysis. The spectra and mass lists were exported to BioTools (version3.1, Bruker Daltonics). Here, both MS and MS/MS spectra were searched against in silico digests of SAM-6 light and heavy chains generated using the Sequence Editor module. The fixed modification of carbamidomethyl-Cys and the optional oxidation of Met or Trp residues were selected prior to the theorctical digest using trypsin with a MS mass tolerance of 50 ppm and a MS/MS tolerance of 0.8 Da. Some ions that were identified as potential sequence variants by LC-ESI-Ion Trap MS(vide infra) were subjected to de novo sequence interpretation to confirm or refute these possibilities.

Liquid chromatography-ESI mass spectrometry (MS and MS/MS)

Sample preparation. One microliter of each in-gel digest was diluted to 5.5 uL with 1% formic acid (FA) in an autosampler vial and 5 uL analysed.

Data acquisition. The samples were chromatographed using an Agilent Protein ID chip column assembly (40 nL trap column with 0.075 x 43 mm C-18 analytical column) housed in an Agilent HPLC Chip Cube Interface connected to an HCT ultra 3D-Ion-Trap mass spectrometer (Bruker Daltonic). The column was equilibrated wtih 4% ACN/0.1% FA at 0.5uL/minute and the samples eluted with an ACN gradient (4%-31% in 32 minutes). Ionizable species (300 <m/z < 1200) were trapped and one of two of the most intense ions eluting at the time were fragmented by collision-induced dissociation(CID) and electron-transfer dissociation (ETD).

Data Analysis. MS and MS/MS spectra were subjected to peak detection using DataAnaylsis (Version 3.4 Bruker Daltonic) then imported into BioTools (Version 3.1, Bruker Daltonic). Here, the MS/MS spectra were matched to the predicted amino acid sequences of the SAM-6 antibody heavy and light chains based on the cDNA sequences obtained from the SAM-6 hybridoma with an MS tolerance of 0.3 Da and an MS/MS tolerance of 0.4Da. The Sequence Editor module was used to calculate the conceptual peptides from the tryptic digestion, including optional modifications such as oxidation of Met and Trp residues, N-terminal pyroglutamic acid and one missed cleavage. In most cases, complementary evidence was obtained from ETD of these peptides. Sequence assignments have been validated manually. Some intense ions that did not match the sequences provded were subjected to de novo sequence interpretation.

Results: The summary of the partial amino acid sequences identified by mass spectrometry are shown in Figures 22 and 23. Data are shown as sequence coverage diagrams wherein grey boxes indicate peptides sequenced and the red boxes indicate the b-ions (upper) and y-ions (lower) observed in CID of these peptides.

SAM-6 Heavy Chain: It was likely that the heavy chain has an *N*-terminal pyroglutamic residue when compared to the DNA sequence of the isolated cDNA from the SAM-6 hybridoma. An ion of $[M+H]^+ = 1592{\cdot}8$ was found with a CID spectrum that matched the expected sequence with this modification. This *N*-terminal modification was then included as a fixed modification in Sequence Editor. The Sequence Coverage diagram is shown as Figure 22.

A peptide that encompasses CDR-H1 was observed. This contains a Cys, not Arg, at position 22 (34 in original numbering). The sequence is therefore 20-LSCAASGFTFSSYAMHWVR-38.

Most of CDR-H2 was observed, as part of two overlapping peptides with sequences; 44-GLEWVAVISYDGSNK-58; and 44-GLEWVAVISYDGSNKYYADSVK-65 with the regions corresponding to CDR-H2 underlined. The sequence observed for CDR-H2 was 50-VISYDGSNKYYADSVK-65 although the SVK tripeptide was not formally assigned. The *C*-terminal residue of the CDR, Gly-66, would have been on a separate (di-)peptide and was not observed. T *versus* A at position 71 (83 in original numbering) was not observed as this peptide was too small to be readily detected.

For CDR-H3, no sequence coverage was seen for this. Both the tryptic and trypsin/V8 digests were not expected to give rise to peptides of mass-to-charge ratios that fell within the operating range of the Ion Trap mass spectrometer. Although the LC-MALDI MS studies addressed a higher MW range than the Ion Trap MS, the predicted tryptic peptide of interest was greater than

6·6 kDa and not of a mass expected to be found readily. Manual inspection of MALDI MS spectra did not identify this peptide or any others of similar MW. Similarly, no peptide corresponding to CDR-H3 was found using LC-MALDI MS of the trypsin/V8 double digest.

SAM-6 Light Chain: Tryptic digests of the SAM-6 light chain were performed in-gel and in solution and analysed by LC-ESI-IonTrap MS and by LC-MALDI-MS. No peptide was identified that corresponded to the predicted *N*-terminus ($[M+H]^+$ for S[1-26]K = 2666.3 and for S[1-30]K = 3079.5). Instead, a peptide of $[M+H]^+$ = 3022.6 was observed that yielded a sequence tag that matched it to the *N*-terminal region. An initial sequence assignment of 1-YE-2 in place of 1-SYV-3 was deduced. The overall sequence coverage is shown in Figure 23. This includes the V126A substitution described below.

The *N*-terminal tryptic peptide was deduced to be 1-YELTQPPSVSVSPGQTASITCSGDK-25. The sequence 1-YEL-3 is commonly found in antibody sequences but not permutations of these residues or the substitution of Leu by Ile. Residues S[22-25]K, which form the *N*-terminal part of the CDR-L1, were consistent with expectation. The longer peptide, Y[1-29]K had a mass and CID spectrum consistent with the predicted sequence for residues L[26-29]K within CDR-L1.

From the in-gel tryptic digest of a peptide with the deduced sequence 30-YACWYQQKPGQSPVLVIYQDSK-52 was found. The underlined sequence corresponds to the *C*-terminal portion of CDR-L1 whilst the double-underlined sequence corresponds to the *N*-terminal portion of CDR-L2. Further support for the predicted sequence of CDR-L2 was obtained from a longer peptide with the predicted sequence 30-YACWYQQKPGQSPVLVIYQDSKRPSGIPER-59 although the CID spectrum was unclear for the *C*-terminus. Although LC-ESI-IonTrap MS did not identify the *C*-terminal portion of CDR-L2, LC-MALDI MS did, albeit with limited sequence coverage by MS/MS as indicated in Figure 23.

The tryptic peptide that contains CDR-L3 was not observed, presumably due to its size being greater than 4.3 kDa. The trypsin/V8 double digest did not provide further information on the sequence of CDR-L3. Several additional peptides were identified that did not match the sequence of the light chain when considering only the standard modifications such as oxidation of Met or Trp and S-amidomethylation of Cys. One ion of [M+H]+ = 1703·0 had a sequence tag that matched S[189-203]K but was 9 Da lighter that expected for the S-amidomethylated peptide. Inspection of the CID spectrum suggested that the Cys residue was present as cysteic acid rather that S-amidomethyl Cys (fig. 8a), which would account for the mass difference. However, a

peptide containing the Cys as S-amidomethyl Cys was also found. Thus, only a fraction of this Cys was oxidized.

An ion of [M+H]+ = 1986·1 had a sequence tag that matched A[110-128]K but was 28 Da lighter that expected. From the ETD and LID spectra it was apparent that the peptide was substituted near the *C*-terminus and a V126A substitution was assigned. This substitution is included in the sequence-coverage diagrams (Figure 23).

Example 9

This example includes a description of how amino acid residues are assigned to the three CDRs in heavy chain variable region and the three CDRs for light chain variable region of SAM-6 and a number of representative variant heavy and light chain variable region sequences. CDR positions are predicted based upon the assignments set forth below and the residue numbering according to Kabat.

HEAVY CHAIN V- domain. According to Kabat numbering, definition is as follows:

CDR-H1

Start: approx residue 26 (always 4 after a Cys)
Residue before: always a Cys-xxx-xxx-xxx-
Residue after: always a Trp, typically WV or WI or WA
Length: 10-12 residues

CDR-H2

Start: always 15 residues after end of CDR-H1
Residue before: typically Leu-Glu-Trp-Ile-Gly (LEWIG)
Residue after: Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala (RFT)
Length: 16-19 residues

CDR-H3

Start approx : always residue 33 after end of CDR-H2 (always 2 after Cys)
Residue before : always a Cys-xxx-xxx-xxx (typically Cys-Ala-Arg)
Residue after: always Trp-Gly- xxx-Gly
Length: 3-25 residues

Thus, for **SAM-6 VH: CDR H1 S25-H35; CDR H2 V50-G66; and CDR H3 R98-Y110**, as indicated by the asterisks (*) below. F denotes a framework mutation, and B denotes a mutation in a CDR.

Sequence of SAM-6 VH and representative variants (underlined):

```
                                    ***** ******              * ********** ******
Percivia        QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
Protein         QVQLVESGGG VVQPGR   L SCAASGFTFS SYAMHWVR      GLEWVAV ISYDGSNKYY ADSVK
1BTA1.1         QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA1.2 (B)     QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFAI(70)
1BTA1.3 (n/d)   RLQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA1.4 (F,B)   QVQLVESGGG VVQPGRSLRL SRAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA1.5 (B)     QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAIHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA2.2 (F,B)   EVQLLESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKDRFTI(70)
1BTA2.7 (F)     EVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
SAM-6 old (B)   QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)

                                          ********** ***
Percivia         SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WG
Protein            DNSKNTLY LQMNSLRAED TAVYYCAR
1BTA1.1          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA1.2          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA1.3          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA1.4          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAARPFDY WGQGTLVTVS S(121)
1BTA1.5          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA2.2          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA2.7          SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
SAM-6 OLD(KT)    SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGKTFDY WGQGTLVTVS S(121)
```

LIGHT CHAIN V-domain. According to Kabat numbering, definition is as follows:

CDR-L1

Start: approx residue 24

Residue before: always a Cys

Residue after: always a Trp, typically WYQ or WLQ or WFQ or WYL

Length: 10-17 residues

CDR-L2

Start: always 16 residues after end of L1

Residue before: generally a Ile-Tyr or VY or IL or IF

Length: always 7 residues (except 7FAB)

CDR-L3

Start approx: always residue 33 after end of L2

Residue before: always a Cys

Residue after: always Phe-Gly- xxx-Gly

Length: 7-11 residues

Thus, for **SAM-6 VL: CDR L1 S23-C33; CDR L2 Q49-S55; and CDR L3 Q88-V96** as indicated by the asterisks (*) below. F denotes a framework mutation, and B denotes a mutation in a CDR.

Sequence of **SAM6 VL** and representative variants (underlined):

```
                                      ******** ***                 **
Percivia        SYVLTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
Protein         -YELTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
1BTA1.6         SYVLTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
```

```
1BTA2.1 (F)  QSVLTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
1BTA2.6 (F)  SYELTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)

         *****                                      *** ******
Percivia SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
Protein  SKRPSGIPER
1BTA1.6  SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
1BTA2.1  SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
1BTA2.6  SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
```

"Percevia" refers to the sequences used for recombinant IgM antibody expression by the PerC6 cell line (Percivia) PAT-SAM-6. "Protein" refers to the amino acid sequence determined by peptide sequence analysis of the antibody produced by the hybridoma cell line (HAB), as described in Example 8. "SAM-6 old" is heavy chain variable region sequence as set forth herein as SEQ ID NO:15.

Additional amino acid and nucleotide sequences of SAM-6 and representative variant sequences are as follows:

SAM-6 defined full length V domain sequences (variants positions underlined):

SAM-6 1.1 VH
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.2 VH
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFAISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVS
S
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCGCCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.4 VH
QVQLVESGGGVVQPGRSLRLSRAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAARPFDYWGQGTLVTVSS

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCCGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT

ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGCTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.5 VH
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAIHWVRQAPGKGLEWVAVISYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATACACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 KT VH (SAM-6 old)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGKTFDYWGQGTLVTVS
S
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAAAACTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 2.2 VH
EVQLLESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKDRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGACCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCAG

SAM-6 2.7 VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.1A imp VH

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 Opt VH 76/363
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVAVISYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGGTCGAGAGCGGGGGAGGCCTGGTCCAGCCAGGGGGATCTCTGAGACT
GAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAGCTGGGTGCGCCAGG
CTCCAGGGAAAGGACTCGAATGGGTGGCCGTGATCAGCTACGACGGCAGCAACAAGTAC
TACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCT
GTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCGGTGTACTACTGCGCCAGGG
ACCGGCTGGCCGTGGCCGGCAGACCCTTCGACTACTGGGGCCAGGGCACCCTGGTGACC
GTGTCCTCT

SAM-6 KT imp VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGKTFDYWGQGTLVTVS
S
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAAAACTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.6 VL (Used in all "A" scFv constructs, e.g., 1.1A scFv has this light chain sequence.)
SYVLTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSG
SNSGNTATLTISGTQAMDEADYYCQAWDSSIVVFGGGTKLTVLGQ

TCCTATGTGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCAGCATC
ACCTGCTCTGGAGATAAATTGGGGGATAAATATGCTTGCTGGTATCAGCAGAAGCCAGG
CCAGTCCCCTGTGCTGGTCATCTATCAAGATAGCAAGCGGCCCTCAGGGATCCCTGAGCG
ATTCTCTGGCTCCAACTCTGGGAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTAT
GGATGAGGCTGACTATTACTGTCAGGCGTGGGACAGCAGCATTGTGGTATTCGGCGGAG
GGACCAAGCTGACCGTCCTAGGTCAGA

SAM-6 2.6 VL (Used in all "B" scFv constructs, e.g., 1.1B scFv has this light chain sequence.)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGS
NSGNTATLTISGTQAMDEADYYCQAWDSSIVVFGGGTKLTVLGQ

TCC TAT GAA CTG ACT CAG CCA CCC TCA GTG TCC GTG TCC CCA GGA CAG ACA GCC
AGC ATC ACC TGC TCT GGA GAT AAA TTG GGG GAT AAA TAT GCT TGC TGG TAT
CAG CAG AAG CCA GGC CAG TCC CCT GTG CTG GTC ATC TAT CAA GAT AGC AAG
CGG CCC TCA GGG ATC CCT GAG CGA TTC TCT GGC TCC AAC TCT GGG AAC ACA GCC

ACT CTG ACC ATC AGC GGG ACC CAG GCT ATG GAT GAG GCT GAC TAT TAC TGT CAG GCG TGG GAC AGC AGC ATT GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT CAG

SAM-6 Opt VL (Kappa) 123/324
DIQMTQSPSSLSASVGDRVTITCRSGDKLGDKYAWYQQKPGKAPKLLIYQDSKHPSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQAWDSSIVVFGQGTKVEIKR

GAC ATC CAG ATG ACC CAG AGC CCC AGC AGC CTG TCC GCC AGC GTG GGC GAC AGA GTG ACC ATC ACC TGC AGA AGC GGC GAC AAG CTG GGC GAC AAG TAC GCC TGG TAT CAG CAG AAG CCC GGC AAG GCC CCC AAG CTGCT G ATC TAT CAG GAC AGC AAG CAC CCC AGC GGC GTG CCC AGC CGG TTT AGC GGC AGC GGC TCC GGC ACC GAC TTC ACA CTG ACC ATC TCC AGC CTG CAG CCC GAG GAC TTC GCC ACC TAC TAC TGT CAG GCC TGG GAC AGC AGC ATC GTG GTG TTC GGC CAG GGC ACC AAG GTG GAG ATC AAG CGG

Example 10

This example includes a description of producing single chain variable region fragments of SAM-6 antibody heavy and light chain variable regions, and representative variant heavy and light chain variable region sequences, and LDL binding and expression studies of the variants.

The construction is done it two parts, the SM-6 VH domain (1BTA1.1-1.6) and then the SM-6 VL domain (1BTA1.6), which can then be joined together and cloned as a Sfi-BglII fragment into the pPOW expression vector. Both parts can be started at the same time.

Sequencing revealed that there are different SAM-6 VH domains which result in amino acid changes. Thus, several scFv constructs with the different sequences were made. Clone 1BTA1.3 has two changes at the start of the VH domain but they are most likely to be errors in construction. Thus, this clone was not used in scFv construction.

For SAM-6 VH (Part A) (use 1BTA1.1, 1BTA1.2, 1BTA1.5), assemble a 20ul PCR reaction with the following components: DNA template, SAM-6 VH SfiI Fwd primer (565897), SAM-6 VH BamHI Rev primer(565352), 10x reaction buffer, dNTP, water to 20ul, and Phusion polymerase. Cycle 95°C x 1 min, 68°C x 30sec, 72°C x 45 sec. Run 5ul on a 1% agarose gel. A band of ~400bp should be seen. Keep the remainder to assemble the ScFv by joining with the VL domain.

For SAM-6 VL (Part B) (use 1BTA1.6), assemble a 20ul PCR reaction with the following components: 1BTA1.6 DNA template, SAM-6 VL BamHI Fwd primer (565354), SAM-6 VL Bk BglII primer (565565), 10x reaction buffer, dNTP, water to 20ul and Phusion polymerase. Run

5ul on a 1% agarose gel. A band of ~400bp should be seen. Keep the reminder to assemble the ScFv by joining with the VH domain.

For SAM-6 ScFv (VH linked to VL), assemble a 20ul PCR reaction with the following components: Part A PCR product (5ul), Part B PCR product (5ul), SAM-6 VH SfiI Fwd primer (565897), SAM-6 VL Bk BglII primer (565565), 10x reaction buffer, dNTP, water to 50ul and Phusion polymerase. Precipitate the DNA with 1/10th Volume Sodium Acetate (3M Na Acetate ph 4.5) and 2 volumes of 100% ethanol. Spin at 14,000rpm for 60 mins. Discard supernatant and resuspend the pellet in 10ul of sterile water.

Set up DNA digest of the PCR product for sub-cloning: 10ul ScFv DNA in sterile water, 1ul 10x NEBuffer 2, 1ul BSA, 1ul SfiI enzyme, and incubate at 50oC for 1 hour. Cool reaction to 37oC, then add 1.5ul of 10x NEBuffer 3, 5ul sterile water, 1ul BglII enzyme and incubate at 37oC for 1 hour. Run all of the digest on a 1% agarose gel. A band of ~800bp should be seen. Cut out the band and extract the DNA using a Qiagen kit.

SAM-6 (VH-5-VL) scFv

```
 Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L   R   L
CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG AGA CTC
 S   C   A   A   S   G   F   T   F   S   S   Y   A   M   H   W   V   R   Q   A
TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT GCT ATG CAC TGG GTC CGC CAG GTC
 P   G   K   G   L   E   W   V   A   V   I   S   Y   D   G   S   N   K   Y   Y
CAG GCA AGG GGC TGG AGT GGG TGG CAG GTT ATA TCA TAT GAT GGA AGC AAT AAA TAC TAC
 A   D   S   V   K   G   R   F   T   I   S   R   D   N   S   K   N   T   L   Y
GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT
 L   Q   M   N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   R   D   R
CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT GCG AGA GAT CGG
 L   A   V   A   G   R   P   F   D   Y   W   G   Q   G   T   L   V   T   V   S
TTA GCA GTG GCT GGT AGA CCT TTT GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC
 S   G   G   G   G   S   S   Y   V   L   T   Q   P   P   S   V   S   V   S   P
TCA GGC GGC GGC GGA TCC TCC TAT GTG CTG ACT CAG CCA CCC TCA GTG TCC GTG TCC CCA
 G   Q   T   A   S   I   T   C   S   G   D   K   L   G   D   K   Y   A   C   W
GGA CAG ACA GCC AGC ATC ACC TGC TCT GGA GAT AAA TTG GGG GAT AAA TAT GCT TGC TGG
 Y   Q   Q   K   P   G   Q   S   P   V   L   V   I   Y   Q   D   S   K   R   P
TAT CAG CAG AAG CCA GGC CAG TCC CCT GTG CTG GTC ATC TAT CAA GAT AGC AAG CGG CCC
 S   G   I   P   E   R   F   S   G   S   N   S   G   N   T   A   T   L   T   I
TCA GGG ATC CCT GAG CGA TTC TCT GGC TCC AAC TCT GGG AAC ACA GCC ACT CTG ACC ATC
 S   G   T   Q   A   M   D   E   A   D   Y   Y   C   Q   A   W   D   S   S   I
AGC GGG ACC CAG GCT ATG GAT GAG GCT GAC TAT TAC TGT CAG GCG TGG GAC AGC AGC ATT
 V   V   F   G   G   G   T   K   L   T   V   L   G   Q
GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT CAG
```

Primers for generation and sequencing of SM-6 PCR product (scFv - dimer):

1) 565897 SM-6 VH Fwd NcoI: 5' AG CCG GCC ATG GCC CAG GTG CAG CTG GTG GAG TCT G 3'
2) 565352 SM-6 VH Bk BamHI GGGGS SYVLTQ: 5' CTGAGTCAGCACATAGGAGGATCCGCCGCCGCCTGAGGAGACGGTGACCAGGGT TCC 3'

3) 576792 PelB extender SfiI: 5'
GCGGGCCTGCTGCTGCTGGCGGCCCAGCCGGCCATGGCC 3'
4) 565354 SM-6 VL Fwd BamHI GGGGS SYVLTQ: 5'
GGCGGCGGCGGATCCTCCTATGTGCTGACTCAGCCACCC 3'
5) 565355 SM-6 VL Bk NotI: KLTVLGQ: 5'
CTTAGCGCGGCCGCCTGACCTAGGACGGTCAGCTT 3'
6) 565565 SM-6 VL Bk BglII: 5'GTCGTCATATCTCTGACCTAGGACGGTAGCTTG 3'
7) 565096 SM-6 VH internal Fwd: 5' GACACGGCTGTGTATTACTGT 3'
8) 565097 SM-6 VL internal Fwd: 5' ACCCAGGCTATGGATGAGGCTGA 3'
9) 575398 SM-6 VH seq rev: 5' TGA GGA GAC GGT GAC CAG 3'
10) 579786 SM-6 VL rev seq: 5' CCTGAGGGCCGCTTGCTATC 3'
11) 577034 SM-6 2.2 Fwd: 5' CAGCCGGCCATGGCCGAGGTGCAGCTGTTGGAGTCT 3'
12) 577142 SM-6 VL SYEL fwd: 5'
GGCGGCGGATCCTCCTATGAACTGACTCAGCCACCC 3'
13) 577131 SM-6 VH EVQLV 2.7fwd: 5'
CAGCCGGCCATGGCCGAGGTGCAGCTGGTGGAGTCT 3'
14) 582061 SM-6 BstEII VL Fwd QSVLT: 5'
GGAACCCTGGTCACCGTCTCCTCAGGCGGCGGCGGATCCCAGTCTGTGTTGACG
3'
15) 633792 SM-6 Opt VH extend NcoI: 5'
GGGCACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGCCGAGGTGCAGCTGGT
CGAGAGCGGGGGAGG 3'
16) 633793 SM-6 Opt VH sequencing NcoI: 5' CCATGGCCGAGGTGCAGCTGG 3'

Primers for generation and sequencing of SM-6 PCR product (scFv - monomer):

17) 619613 SM-6 VLA 15aa mono:
5' GGAACCCTGG TCACCGTCTC CTCAGGCGGC GGCGGAAGCG GCGGCGGCGG
ATCTGGCGGC GGCGGAAGCT CCTATGTGCT GACTCAGCCA 3'

Primers for generation and sequencing of SM-6 PCR product (IgG):

18) 617413 VH IgG leader AscI build IRRAATMACPGFLWALVIST: 5'
ATTCGGCGCGCCGCCACGATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCT
CCACC 3'
19) 620112 VH domain IgG leader AscI: 5' ATTCGGCGCGCCGCCACGATGGCATG 3'
20) 617411 VH3 Fwd Sfi: 5'
CTGGCGGCCCAGCCGGCCATGGCCGAAGTGCAGCTGGTGGAAAGC 5'
21) 616285 VH doman Rev IgG constant: 5' TTGGTGCTAGCTGAGGAGACGGTGAC 3'

22) 620049 VH KT opt II build EVQLVES: 5'
GGGCACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGCCGAGGTGCAGCTGGT
GGAGTCT 3'
23) 620113 SM-6 VH domain ApaI rev: 5'
GAAGACCGATGGGCCCTTGGTGCTAGCTGAGGAGACGGTGAC 3'
24) 620107 HC Rev IgG HpaI New: 5' CTGTCCGTTAACTCATTTACCCGGAGA 3'
25) 633471 EVQLVES Per SM-6 imp seq: 5' GAGGTGCAGCTGGTGGAGTCT 3'
26) 633794 SM-6 IgG VH rev: 5'
CCGATGGGCCCTTGGTGCTAGCAGAGGACACGGTCACCAGGGTG 3'
27) 633795 SM-6 Opt VH Fwd HC: 5'
CACCCTGGTGACCGTGTCCTCTGCTAGCACCAAGGGCCCATCGG 3'
28) 633814 SM-6 Opt VLk seq F: 5' GAATTCAGCATGGCCGACATCC 3'
29) 633815 SM-6 Opt VLk seq Rev: 5'
CACGCTGGGAGCGGCCACGGTCCGCTTGATCTCCACCTTGGT 3'
30) 633861 SM-6 SEQ F: 5' CAAGAACACCCTGTACCTGCA 3'

A summary of nine scFv variants produced and expressed from bacteria using the pPOW vector, and the heavy and light chain variable region sequences they contain are listed below:

SAM-6 1.1A scFv: The 1.1A scFv construct has the same V domain gene sequence as the PAT-SAM-6 IgM (Percivia) gene construct.
SAM-6 1.2A has a single aa change in VH CRD2, and same VL domain as 1.1A
SAM-6 1.4A has 1 framework and 1 VH CRD3 change, and same VL domain as 1.1A
SAM-6 1.5A has 1 VH CDR1 aa change, and same VL domain as 1.1A
SAM-6 2.2A has 2 framework and 1 VH CRD2 changes, and same VL domain as 1.1A
SAM-6 2.7A has 1 framework aa change, and same VL domain as 1.1A
SAM-6 KTA: The main difference between the KTA scFv construct and the 1.1A is that it has 2 amino acid changes in the VHCDR3 binding loop. This binding loop is believed to be the most important region of an antibody as it is in this region that the majority of binding reactions take place. The antigen specificity is attributed to this region. The amino acid sequence in the KTA construct is the same as was originally reported in the first SAM-6 patent application. It is the protein in the SAM-6 "family" that still uses heavy chain variable region amino acid sequence, SEQ ID NO:15.
PAT-SAM-6 (Percivia) IgM: Recombinant antibody produced in PerC6 cells (Percivia). There is an amino acid change in the VL domain framework. This change from a V (Valine) to an E (Glutamic acid) appears to improve protein expression levels by the cells. SAM-6 HAB IgM:

Human hybridoma produced protein (Patrys GmBH, Germany). The gene sequence for this protein differs from the PAT-SM-6 IgM in the VL domain, based on protein sequence by Mass Spec.

SAM-6 1.1B scFv has a the same VH sequence as 1.1A, but a single amino acid change in the VL domain

SAM-6 2.2B scFv has 2 framework and 1 VH CRD2 changes, and single amino acid change in the VL domain

Binding to LDL was used to measure protein expression of the various scFv sequences by bacteria. The data reveal that the strongest signal is observed with the SAM-6 KTA and SAM-6 2.7, indicating that these two variants were produced in higher amounts. The variant residues in each will be combined to incorporate these amino acid changes in an IgG variant of SAM-6.

Example 11

This example includes a description of binding studies showing that SAM-6 diabodies bind to an antigen in conditioned media from A549 cells, and bind to LDL (low density lipoprotein). This example also includes data indicating that certain SAM-6 variants are produced in greater amounts when expressed in cells.

In the ELISA (Figure 24), the two bars to the right show binding of scFv 1.1A and 1.1B to LDL. In brief, LDL is coated onto the plate, then the plate is blocked. SAM-6 diabodies (with FLAG tag at C-terminus) are added (incubated) then the unbound protein is washed away. Another antibody is added that binds to the FLAG tag that is also coupled to HRP (HorseRraddish Peroxidase). This binding can be detected in a colour reaction that is recorded by the ELISA plate reader. An absorbance reading around 1.0 indicates that the proteins are binding.

SAM-6 binds to the cancer cell line A549, so these cells produce target in an accessible form that the antibody binds to, most likely on the cell surface. A549 cells were grown and by day three the cells have formed a confluent layer on the bottom of the tissue culture flask. Spent culture media (now called conditioned media as it now has the A549 cell growth by-products in it, including fetal calf serum– as well as the secreted target protein, but no A549 cells as they are removed by centrifugation) were collected and coated to ELISA wells. The neighbouring (control) well had growth media that never had any cells growing in it. After blocking, the SAM6-diabodies are added and allowed to bind. Unbound protein is washed away and the secondary antibody added for flag tag detection.

The two conditioned media bars show that A549 cells produce material in the medium to which SAM-6 antibody binds (Figure 24). The A549 cells produced an antigen target that is apparently secreted into the culture media. This data indicates that there is a large amount of target (antigen) protein in the A549 conditioned media.

The binding to LDL data indicate that scFv KTA (SAM-6 old) with two residue change in CDR3 had greater relative binding to LDL than other SAM-6 variants. The data also reveal that a change of the framework region in scFv 2.7A also had greater relative binding affinity for LDL than other SAM-6 variants. A summary of the rank order for binding to LDL for scFv variants was:

KTA (SAM-6 old) better than 1.1A (B, CDR3)
2.7A better than 1.1A (F)
1.1A = Percivia expressed (PAT-SAM-6)
1.1B less binding than 1.1A (F)
2.2B less than 1.1A (F,B)
1.5A less than 1.1A (B)
1.4A less than 1.1A (F,B, CDR3)
1.2A less than 1.1A (B)
2.2A less than 1.1A (F,B)
(F)= Framework change; and (B)= Binding region change

Thus, combining the KTA (SAM-6 old) and the 2.7A framework should result in improved binding affinity for LDL.

Example 12

This example includes a description of studies showing that SAM-6 diabodies bind to an antigen in conditioned media from A549 cells, and bind to a cell free translated non-glycosylated grp78 protein (using wheat germ).

ELISA assays were performed. Antigen was coated at 0.5 µg/well in a volume of 50µl/well. Primary antibody was used at 12µg/ml (0.6µg/well). Buffer was pH6.5 and the dilution buffer used was a high salt pH6.5. Grp78 protein was diluted with 1 x PBS pH7.4

ELISA demonstrated binding to a component of A549 cancer cell culture media (after cells were grown for three days). A549 conditioned media (cells removed) was coated onto the entire plate. The SAM-6 diabodies were added (1.1 and KTA and optimised). Other control antibodies were

included, an LM1 diabody, CM1 diabody, a control VH dimer-which is actually a monomer, a BARB3-diabody, BARB4 diabody, a recombinant PAT-SAM6 450-IgM (produced by PerC6 cells, Percivia), recombinant LM1 41B1-IgM , and SAM6 C8/9 hybridoma IgM. The negative controls are the conditioned media that the A549 cells have been growing in, without the primary antibody and with the secondary antibody. The data show that all of the SAM-6 diabodies bind to an antigen present in the A549 culture supernatent, it is also detected by the recombinant SAM-6 IgM clone 450 (produced by PerC6 cells, Percivia). SAM-6 hybridoma C8/9 gives a very poor signal, but this may be due to protein degradation or hybridoma cell death. This ELISA also shows that the other antibodies tested do not bind to any secreted product in the A549 conditioned media. Only SAM-6 detectably binds to the A549 conditioned media. This ELISA shows that SAM-6 binds to a target in A549 cell conditioned media.

Conditioned media from a second cell line HDFa previously shown not to exhibit cell surface binding to SAM-6 antibodies was studied for binding to SAM-6 antibodies. No binding was detected indicating that this cell line is a good negative control.

Another ELISA was performed on a plate coated with Grp78 protein (from Abnova- cell free protein translation-using wheat germ-non-glycosylated). Binding of recombinant PAT-SAM-6 IgM antibody (clones 450 and 528 produced by PerC6 cells, Percivia) and recombinant SAM-6 1.1A diabody to pure non-glycosylated Grp78 protein was detected. All binding was to pure (non-glycosylated) target Grp78 protein. This data indicates that SAM-6 antibodies and variants bind to grp78 without a carbohydrate moiety. On the second half of the plate binding to conditioned media from A549 cells was detected, whereas negative control LM1 antibodies did not bind to the conditioned media. There was variation in the strength of the signal detected but the target protein may not be uniformly dispersed throughout the sample.

Example 13

This example includes a description of studies showing various forms of SAM-6, including SAM-6 scFv, SAM-6 variants and SAM-6 heavy chain variable region (V_H) alone, without light chain variable region (V_L) bind to an apoB100, protein, LDL, VLDL and deglycosylated LDL.

Antigen specificity: Fresh batches of recombinant protein were made and tested against a panel of proteins to determine specificity for LDL. The ELISAs were repeated several times. Positions of the antigens on the plates were randomized to rule out position effects.

For SAM-6 KTA scFv, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 KTA scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). Note that in these protein samples the soluble B fraction has been removed and only the remaining soluble C fraction is tested. The 3rd time reading are higher as they contain the combined protein level (1CHO4.8) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49). The well contains the antigen, Lewis Y tetrasaccharide bound to HSA(Human Serum Albumin). The positive control (anti-Lewis Y) gave an absorbance reading at A655nm of 0.98 on one ELISA and 0.90 on the other ELISA when binding to its carbohydrate antigen lewis Y.

The strongest binding of SAM-6 KTA scFv is to Apolipoprotein B100. Binding to VLDL, LDL and deglycosylated LDL was also detected.

For SAM-6 1.1A scFv urea solubilized, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 1.1A scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). The 3rd time readings contain the combined protein level (1CHO4.7) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49) gave an absorbance reading at A655nm of 1.2 and 1.0.

Strong binding of SAM-6 1.1A to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 (Percivia), strong binding to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 HAB produced by human hybridoma (Patrys GmBH, Germany), strong binding to VLDL, LDL and de-glycosylated LDL, was detected but less binding to apolipoprotein B100. The SAM-6 HAB gave variable results in this assay.

In the foregoing studies several different SAM-6 proteins produced in a variety of different formats were compared for their ability to bind to various target antigens, such as LDL (Low Density lipoprotein), VLDL, deglycosylated LDL and apoB100 protein. SAM-6 KTA scFv, 1.1 scFv, PAT-SAM-6 (Percivia) and SAM-6 HAB exhibited various degrees of binding affinity for LDL, VLDL, deglycosylated LDL and apoB100 protein, but not HDL (high density lipoprotein). In this way sequence changes can be linked to function.

Further binding studies to ApoB100 were performed by ELISA analysis. In brief, 250ul of Apolipoprotein B100 (10ug/ml) isolated from low density LDL (purchased from Calbiochem) was coated onto ELISA plates. Plates were blocked, incubated with primary single-chain antibodies (SAM-6.2.7 and SAM-6.opti) and SAM-6 heavy chain variable region (V_H) alone), and then incubated with anti-FLAG-HRP secondary antibody in a total volume of 250ul, and compared to three negative controls (Negative control 1: No coating (blocked), No primary, then anti-FLAG-HRP secondary; Negative control 2: No coating (blocked), then primary, then anti-FLAG-HRP secondary; and Negative control 3: Coated with 10ug/ml ApoB100 (blocked), No primary, then anti-FLAG-HRP secondary).

The results indicated that SAM-6.2.7, SAM-6.opti and SAM-6 heavy chain variable region (V_H) alone) bind to ApoB100 protein.

Example 14

This example includes a description of studies showing that SAM-6 variants can also bind to cancer cell lines A549, BxPC3 and CRL1424.

The following variants were studied: SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia). SAM-6 VHVL opt scFv has an optimized framework with 4 amino acid changes in the VH domain including 25% changes at the nucleotide level. There is one additional change in CDR-H1. The VL domain of SAM-6 VHVL opt scFv is a class switch from lambda to kappa light chain with 40 amino acids changed including 38% changes at the nucleotide level. The free Cys residue was removed from the VL CDR1.

FACS analysis revealed that all of the scFv constructs bind to the three cancer cell lines tested (A549, BxPC3 and CRL1424), but not to the negative cell line HDFa.

Additional studies were performed using confocal microscopy analysis for binding to A549, BxPC3, CRL1424, HT-29, HeLa, and MCF-7 cancer cell lines. The SAM-6 antibodies studied included SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia).

In brief, cells were fixed, the primary antibody was added, then detected with a secondary antibody with a FITC label. The cell nucleus was stained with a DAPI stain that appears blue, and measured in the 600-650 wavelength range. This DAPI image was captured and recorded. If the level of DAPI nuclear stain was kept at a constant level, different studies can be "normalized." The cells were incubated with the primary (test) antibody, and the appropriate labelled secondary

antibody added. In these experiments we used an FITC label that was measured at the maximum intensity observed in the 500-550 wavelength range. The FITC image was recorded. The images were overlays of the DAPI image and the FITC images.

In another set of studies the ability of the proteins to show two active binding sites was studied. In order to see a positive result, the protein would need to bind to the cell surface target antigen on the cancer cell line, with one binding arm, and then the second arm would be required to bind to the human LDL labelled with Alexa 488. Only when these two events occur would a binding event be detected. The Alexa 488 image does not fade and is more stable and we found that it generated a more intense staining image.

The results revealed binding was detected for A549 (Lung), BxPC-3 (pancreatic), HT-29 (colon), HeLa (cervix), MCF-7 (breast) and CRL1424 (melanoma) cells for all five SAM-6 antibodies. Differences were observed in the binding of the SAM-6. In some cell lines it appears that the SM-6 proteins have entered the cell nucleus, when the overlays are done the cell nucleus appears a lighter but brighter blue. None of the five SAM-6 antibodies detectably bind to stomach cancer cell line 23132/93.

Example 15

This example includes a description of additional studies with IgG1 SAM-6 variants, which can also bind to cancer cell line A549.

SAM-6 scFv's were converted into IgG1 using the lambda light chain and the IgG protein expressed in mammalian cells HEK293F. To date, the three different SAM-6 proteins produced in IgG1 format are SAM-6 1.1imp IgG, which contains the 1.1A VH domain with one amino acid change to improve expression, now called 1.1imp. It also contains the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinaint IgM construct. SAM-6 KT imp IgG, which contains the KTA VH domain with one amino acid change to improve expression and the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinaint IgM construct. SAM-6 opt IgG, which contains the S6 optimised VH domain with four amino acid changes as well as codon optimisation to improve expression and the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinant IgM construct. KTA means that the KT VH is paired with the 1BTA1.6 VL domain in the scFv construct.

Culture supernatant was isolated after transient transfection of HEK293F cells. At day 5, cell supernatant with the appropriate lgG1. Binding of SAM-6 1.1imp lgG1, SAM-6 KT lgG1 and SAM-6 opt lgG1 to A549 cells was detected.

Additional FACS studies were performed with HeLa cells with SAM-6 1.1imp lgG1 and SAM-6 Kopti lgG1. Binding of SAM-6 1.1imp lgG1 and SAM-6 Kopti lgG1 to HeLa cells was detected.

Example 16

This example includes a description of studies showing that alterations of amino acid residues in SAM-6 scFvs can increase protein solubility.

The limited solubility of scFv's antibodies, as seen with SAM-6 1.1A, whose Fv sequence is unchanged from parent PAT-SAM-6 lgM, has limited storage and reduced efficiency in trials of the antibody over time. However, as shown in the Table below, with SAM-6 optimized (scFv dimmer) and SAM-6 opt. scFv monomer, codon usage optimization combined with targeted residue changes in the VH domain as well as a new VL domain framework all contribute to improved protein solubility.

Solubility of scFv's in Biological Buffers

scFv antibody	Concentration from Profinia™ (containing 6M Urea)	Concentraion post dialysis (BCA)	Percent recovery after dialysis
SAM-6 1.1A	1.100 mg/ml	0.174 mg/ml	16%
Optimized dimer	1.200 mg/ml	0.861 mg/ml	72%
PAT-SAM-6 opt monomer	1.300 mg/ml	1.223 mg/ml	94%

The data in the table indicate that SAM6 optimized (diabody) vs SM-6 opt (optimized) monomer have a higher yield than SAM-6 1.1A.

Example 17

This example includes a description of studies showing ELISA binding studies of SAM6-IgM to lipoprotein (LDL) and an apoptosis assay.

PAT-SAM6-IgM shows binding to LDL relative to isotype matched human IgM antibody (Figure 25A). Furthermore, binding of PAT-SAM6-IgM is increased after Cu2+ oxidation of LDL (Figure 25A). Antibody-induced lipoptosis of tumor cells in the presence of differently Cu-oxidized LDL was measured by Cell Death Detection ELISAPLUS. Pancreatic carcinoma cell line BXPC-3 was incubated with PAT-SAM6-IgM and unrelated human IgM isotype control. Amounts of apoptotic cells were determined photospectrometrically at 415 nm (reference λ 490 nm). PAT-SAM6-IgM ability to induce lipoptosis/apoptosis is enhanced in the presence of increased Cu^{2+} oxidized LDL (Figure 25B).

Example 18

This example includes a description of studies showing SAM-6 immunoprecipitation of target antigen, and possibly antigens associated with target antigen, from conditioned media produced by A549 cells.

Immunoprecipitation studies of conditioned media from A549 cells with SAM-6 diabody was performed. The immunoprecipitated portion was fractionated on a10% SDS-PAGE and subsequentely silver stained. As illustrated in Figure 26, SAM-6 diabody binds to several proteins around 110 to 50 kDa, as well as lower molecular weight proteins, present in the A549 cell conditioned media. These proteins may be a SAM-6 target, a target fragment or a protein that is associated with a SAM-6 target. The 30 kDa is presumed to be the SAM-6 diabody.

Example 19

This example includes a description of additional studies showing increased affinity of a particular SAM-6 variant (optimized scFv dimer) for LDL, as compared to SAM-6 1.1A scFv and SAM-6 optimized scFv monomer.

PAT-SAM-6-IgM was previously been shown to bind LDL and induce lipoptosis in cancer cells. Affinity of other PAT-SAM-6 scFv variants to LDL was assessed via ELISA. All variants bind positively to LDL, with PAT-SAM-6 scFv diabody optimized having the greatest affinity.

Example 20

This example includes a description of additional SAM-6 binding studies, and that SAM-6 does not bind to CD55 antigen.

In brief, CD55 antigen (0.5 µg/well) was coated in a volume of 50µl/well. The primary antibody was incubated at 12 µg/ml (0.6 µg/well). Buffer was pH6.5 and for the dilution buffer used was High salt pH8.0. These ELISA studies revealed that SAM-6 does not bind to CD55 antigen.

Example 21

This example includes a description of additional SAM-6 binding studies, and that SAM-6 heavy chain variable region (V_H) alone, without light chain variable region (V_L), binds to target.

The nucleotide and amino acid sequences of SAM-6 heavy chain variable region (V_H) alone, respectively, used in the binding studies:

Nucleotide:
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGC
CAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAA
TAAATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAA
GAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTA
CTGTGCGAGAGATCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGG
AACCCTGGTCACCGTCTCCTCA

Protein:
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNK
YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTL
VTVSS

SAM-6 heavy chain variable region (V_H) alone was prepared using the following materials and methods: 1L culture flasks, 50ml Falcon tubes, Petri dishes, Eppendorf Biophotometer or equivalent to read OD_{600} and 280nm, 1.5ml Eppendorf tubes, LB amp agar plates, Bio-Rad 1ml spin columns, Vacuum Manifold, Suspension mixer.

For expression of SAM-6 heavy chain variable region (V_H) protein, prepare fresh transformations of nucleic acid into JM109, inoculate 10ml Super Broth (SB) amp medium with a single colony and grow at 33°C with shaking approximately 150rpm overnight, then dilute overnight 1:10 in SB. Record the culture OD600 (1ml as the blank SB) and then dilute 100µl of the culture plus 900µl SB. Grow culture until it reaches an OD600 of ~4.000. Take 1ml aliquot as the 0hour sample and store at 4°C for later analysis. Shift the cultures to another incubator shaker set at 42°C and continue shaking at approximately 150rpm for 4h. Check progress of induction periodically after 3 hours, once OD has stabilized induction is complete, record the culture OD600 (1ml as the blank) and then dilute 100µl of the culture plus 900µl terrific broth. Take 1ml aliquot at 4hours post-induction, transfer cultures to 50ml Polycarbonate Falcon Tubes (BD). Pellet down (spin at 4000g, 4°C for 20mins) the balance of the induced culture for protein extraction and purification, and perform SDS-PAGE (Coomassie) and α-FLAG Western on the whole cell lysate aliquots and on the purified proteins.

SAM-6 heavy chain variable region (V_H) protein was then extracted by lysing the cell pellet in 2ml BugBuster Master Mix (Novagen) and mixing on a suspension mixer for 20 minutes. The solution was then made to 6M Urea and gently mixed for a further 10 minutes, followed by addition of 600µl of Ni-NTA resin (QIAGEN) and mixing the solution on a suspension mixer for

60 minutes. The protein resin solution was then collected into a 1.0ml disposable spin column (Bio-Rad) under vacuum (10 in Hg) trapping the resin and bound His tagged scFvs in the column, washed with 10ml of IMAC wash buffer, and then bound proteins eluted with 5ml of IMAC elution buffer.

Buffer exchange with eluted proteins was performed with Amicon Ultra-15 devices as follows: Wash devices with buffer to remove preservative on the membranes, usually glycerol (not essential but recommended). Place sample into filter compartment and make to 15ml with Hepes Buffered Saline (HBS), pH7.3 spin at 4000rpm (4°C) for 30 minutes (samples spun at 4°C will take longer than those spun at RT). Once sample is at ~1.0ml discard filtrate and add a further 14ml of HBS, pH7.3, to filter compartment and spin again. Recover protein sample and measure concentration at 280nm with Eppendorf BioPhotometer using BSA standard curve.

Reagents are listed in the following table:

Item	Supplier Of individual reagents	Comments	Storage
Super Broth	Patrys Refer SOP 2.20	Fresh Amp < 3 weeks	4°C
Ampicillin	Sigma	Made to 100mg/ml stocks. 1ml/L Broth	-20°C
BugBuster Master Mix	Novagen		4°C
Ni-NTA resin	QIAGEN		4°C
HBS			
DNA SM6 2.7A	Patrys 2BTA8.4.3		-20°C
DNA SM6opt	Patrys 3LUD39		-20°C
DNA SM6 VH alone	Patrys 1TWE2.3.3		-20°C
IMAC wash buffer	Made by Patrys "inhouse"	50mM KH2PO4, pH 8.0, 300mM KCl, 5mM imidazole, 6M Urea.	4°C
IMAC elution buffer (no Urea)	Made by Patrys "inhouse"	50mM KH2PO4, pH 8.0, 300mM KCl, 500mM imidazole.	4°C
Hepes Bufferd Saline, pH7.3 (HBS)	Made by Patrys "inhouse		

FACS analysis of SAM-6 heavy chain variable region (V_H) protein was performed as follows: 2 x 10^5 cells/ml (per sample) used in ice cold FACS buffer in 1.5ml eppendorf tube and let it sit on ice for 30 mins; for the control cells only, used 4 x 10^5 cells/ml. Centrifuge the tubes containing cells for 5 mins at 500g at 4°C, discard the supernatant, add the primary antibody at neat concentration (total volume 50µl), and leave on ice for 30 mins in the dark. Add 1ml ice cold FACS buffer (30 FACS buffer has 120µl, 2mM EDTA (stock 500mM), 300µl of 1% FBS HI, and 1xPBS, pH7.4, 30ml), and centrifuge for 5 mins at 500g at 4°C, add 2µl of the 2° antibody in 1/20 dilution in ice cold FACS buffer (total volume 50µl), leave on ice for 30mins in the dark. Add 1ml ice cold FACS buffer, centrifuge for 5 mins at 500g at 4°C, resuspend the cells in 200µl of ice cold FACS buffer (for the control cells only tube resuspend in 400µl). Filter through a nylon

filter and transfer the cells to 5ml round bottom FACS tubes (check if there is any cell clumps), and just before analyzing add 1µl of Propidium Iodide (1mg/ml). Mix samples with a vortex mixer, and analyze the samples in the FACS machine. The data was analyzed with Win MDI program.

SAM-6 antibodies and variants used in the studies were SAM-6.2.7.ScFv (2CHO43.1), SAM-6.Opti.ScFv (2CHO43.2) and SAM-6.VH only (2CHO43.3). Cell lines used were HeLa and HDFa cell lines.

FACS buffer was stored on ice. Propidium Iodide (Sigma, 1mg/ml), Polyclonal rabbit Anti-Human IgM/FITC (Dako, Lot no: 00051504), Anti Flag M2 Monoclonal FITC (Sigma, Lot No: 105K62091), Percivia SM-6 (+ J chain) clone 450 were all stored at 4°C.

The results are illustrated in Figure 28, in the top row, 1st Panel- scFv negative controls, 2nd Panel -SM6 2.7A scFv (black line), and 3rd Panel -SM6 opti scFv(black line). In the bottom row, 1st Panel- SM6 VH alone (black line), 2nd Panel –anti-IgM negative control, and 3rd Panel -SM6 IgM 450 (black line).

In Figure 28, the first row (top) there are two examples of SAM-6 single-chains (VH + VL). The SAM-6 2.7 contains the VH and VL using lambda light chain. The SAM-6opti contains the VH and VL using kappa light chain. The next (bottom) row has the VH domain only and exhibits similar binding. The last panel (right) has the SAM-6 IgM binding. The first (left) panel on the second row shows binding of the SAM-6 VH alone to Hela cells. Thus, binding to SAM-6 target can be conferred by the VH alone of SAM-6.

Example 22

This example includes a description of studies showing that SAM-6 variants, bind to a secreted recombinant Grp78 (glycosylated) expressed by mammalian 293 F cells.

Recombinant Grp78 (mammalian secreted, glycosylated, full-length with His x 6 tail) with the KDEL C-terminal sequence to secrete the protein was produced from the mammalian 293F expression system.

ELISA assays were performed. In brief, recombinant Grp78 was coated at 10µg/ml (batch no: TC0903-18), and primary antibody at 100µg/ml. Secondary antibodies were Anti-Flag-M2 Monoclonal HRP Antibody for ScFv SAM-6 and polyclonal rabbit anti human IgM/HRP for SAM-6. Negative controls were 1) Coated with 10µg/ml Grp78, No primary and Anti Flag/HRP secondary; 2) Coated with 10µg/ml Grp78, No primary and Polyclonal rabbit anti human IgM /HRP secondary; 3) No Coating, No Primary, Polyclonal rabbit anti human IgM/HRP secondary only; 4) No Coating, No Primary, Anti Flag/HRP secondary only; 5) No Coating, SAM-6 clone

450 and Polyclonal rabbit anti human IgM/HRP secondary; 6) No Coating, LM1 clone 170 and Polyclonal rabbit anti human IgM/HRP secondary; and 7) No Coating, PAT SM6 2.7 scFV and Anti Flag/HRP secondary. Volume was 250µl. Antibodies studies were Single-chain antibodies of SAM-6 2.7 Flag, Single-chain antibody of SAM-6 Opti (kappa light chain), SAM-6 V_H alone Flag; Flag SAM-6 IgM 450 and LM1 IgM clone 170.

The studies in Figure 29 demonstrate that SAM-6 2.7, Single-chain antibody of SAM-6 Opti (kappa light chain), and SAM-6 V_H alone bind to recombinant Grp78 (glycosylated) expressed by mammalian cells.

What is Claimed is:

1. An isolated or purified antibody that specifically binds to Grp78, wherein the antibody competes with SAM-6 as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) for binding to Grp78.
2. An isolated or purified antibody that specifically binds to apoB100, wherein the antibody competes with SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) for binding to apoB100.
3. An isolated or purified antibody that specifically binds to LDL, VLDL, or oxidized LDL, wherein the antibody competes with SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) for binding to LDL, VLDL, or oxidized LDL.
4. An isolated or purified antibody that specifically binds to deglycosylated Grp78 or deglycosylated LDL, wherein the antibody competes with SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) for binding to deglycosylated Grp78 or deglycosylated LDL.
5. The antibody of claim1, wherein the Grp78 comprises a sequence of about 655 amino acids.
6. The antibody of claim 1, wherein the antibody binds to a Grp78 extracellular or intracellular domain.
7. The glycoprotein of claim 6, wherein the intracellular domain comprises about 411 amino acids and the extracellular domain comprises about 220 amino acids.
8. The antibody of claim 1, wherein treatment of the glycoprotein with a glycosidase enzyme reduces binding of SAM-6 antibody to the Grp78.
9. The glycoprotein of claim 8, wherein the glycosidase enzyme comprises an O-glycosidase.
10. The antibody of claim 1, wherein treatment with endoglycosidase H or endoglycosidase F reduces the apparent molecular weight of the Grp78.

11. A subsequence of the antibody of claims 1 to 4, wherein the subsequence binds to one or more of Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL.
12. The antibody of any of claims 1 to 4, wherein the heavy or light chain variable region comprises a SAM-6 VH or SAM-6 VL sequence as set forth herein.
13. The antibody of any of claims 1 to 4, wherein the antibody has greater binding affinity for Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL than the binding affinity of SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) for binding to Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL.
14. The antibody of claim 1, wherein the Grp78 has at least 60%, 70%, 80%, 90%, 95% or more identity with SEQ ID NO:1, or a subsequence thereof.
15. The antibody of claim 1, wherein the Grp78 is characterized as being expressed or secreted by a neoplastic, cancer or tumor cell, or a pancreas carcinoma cell line or lung carcinoma cell line denoted respectively as BXPC-3 (ATCC Deposit No. CRL-1687) or A549 (DSMZ Deposit No. CCL185).
16. A nucleic acid sequence that encodes the antibody of any of claims 1 to 4.
17. A nucleic acid sequence that encodes the antibody subsequence of claim 12.
18. A nucleic acid sequence that is at least 75-90% or more, complementary or homologous to a nucleic acid sequence that encodes the antibody or a subsequence thereof of any of claims 1 to 4, wherein the nucleic acid sequence has a length from about 10-20, 20-30, 30-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-1000, 1000-2000 nucleotides.
19. A nucleic acid sequence that specifically hybridizes to the nucleic acid sequences of any of claims 16 to 18.
20. The nucleic acid of claim 16 or 17, further comprising an expression control sequence.
21. A vector comprising a nucleic acid that encodes the antibody of any of claims 1 to 4, or the antibody subsequence of claim 12.
22. The vector of claim 21, wherein the vector comprises a viral, bacterial, fungal or mammalian vector.
23. A host cell transformed with the nucleic acid of claim 16 or 17 or vector of claim 21.
24. The host cell of claim 23, wherein the host cell is eukaryotic.
25. The host cell of claim 24, wherein the cell is stably or transiently transformed.

26. An isolated or purified antibody that specifically binds to an antigen or epitope of Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL to which SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) binds.

27. An isolated or purified antibody that specifically binds to a sequence of Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL to which SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) binds.

28. An antibody that inhibits or blocks binding of SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18) to Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL.

29. An antibody inhibits or prevents binding of SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18), to Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL as determined in an ELISA assay, a western blot or an immunoprecipitation assay.

30. An antibody that inhibits at least 50% of the binding of SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18), Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL.

31. The isolated or purified antibody of any of claims 1 to 4 or 26 to 30, wherein the antibody binds to low density lipoprotein (LDL) or oxidized LDL (oxLDL).

32. The isolated or purified antibody of any of claims 1 to 4 or 26 to 30, wherein the antibody binds to a component present in A549 cell conditioned media.

33. The isolated or purified antibody of any of claims 1 or 26, wherein binding of the antibody to cells expressing the Grp78 stimulates or induces cell death, lysis or apoptosis.

34. The isolated or purified antibody of any of claims 1 or 26, wherein binding of the antibody to a neoplastic, cancer or tumor cell expressing the Grp78 stimulates or induces cell death, lysis or apoptosis.

35. The isolated or purified antibody of any of claims 1 to 4 or 26 to 30, wherein binding of the antibody to BXPC-3 or A549 cells inhibits BXPC-3 or A549 cell growth or proliferation, or stimulates or induces BXPC-3 or A549 cell death, lysis or apoptosis.

36. The isolated or purified antibody of any of claims 1 or 26, wherein binding of the antibody to cells expressing Grp78 causes activation of a caspase.

37. The isolated or purified antibody of claim 36, wherein the caspase comprises caspase-3, caspase-7, caspase-8 or caspase-9.

38. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody does not bind to an epitope or sequence comprising an N- or O-linked carbohydrate moiety.

39. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody does not have a heavy or light chain variable sequence identical to SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:18, or antibody produced by hybridoma deposited as DSM ACC2903.

40. The isolated or purified antibody of any of claims 1 to 4 or 26 to 30, wherein the antibody comprises a heavy or light chain CDR3 with at least 100% identity to a heavy or light chain CDR3 set forth herein.

41. The isolated or purified antibody of any of claims 1 to 4 or 26 to 30, wherein the antibody comprises a heavy chain variable sequence with a CDR with 100% identity to CDR3 (ARDRLAVAGRPFDY; SEQ ID NO:17) of heavy chain variable region amino acid sequence set forth as SEQ ID NO:18, or heavy chain variable region of antibody produced by hybridoma deposited as DSM ACC2903.

42. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody comprises a heavy chain variable sequence with 100% identity to a CDR1, CDR2, or CDR3 of a heavy chain variable region amino acid sequence set forth herein.

43. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody comprises a light chain variable sequence with 100% identity to a CDR1, CDR2, or CDR3 of a light chain variable region amino acid sequence set forth herein.

44. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody is polyclonal or monoclonal.

45. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody is selected from IgG, IgA, IgM, IgE and IgD.

46. The antibody of claim 45, wherein the IgG is an IgG1, IgG2, IgG3, or IgG4.

47. The isolated or purified antibody of claims 1 to 4 or 26 to 30, wherein the antibody has a binding affinity for Grp78, apoB100, LDL, VLDL, or oxidized LDL, deglycosylated Grp78 or deglycosylated LDL, within about 1-5000 fold of the binding affinity of SAM-6

antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

48. The antibody of any of claims 1 to 4 or 26 to 30, wherein the antibody has a binding affinity for Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL within about KD 10^{-5} M to about KD 10^{-13} M of SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or comprising a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

49. The antibody of any of claims 1 to 4 or 26 to 30, comprising an antibody subsequence that binds to one or more of Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL, or an antigenic fragment thereof.

50. The antibody subsequence of claim 49, wherein the subsequence is selected from Fab, Fab', $F(ab')_2$, Fv, Fd, single-chain Fv (scFv), disulfide-linked Fvs (sdFv), V_L, V_H, trispecific (Fab_3), bispecific (Fab_2), diabody ($(V_L\text{-}V_H)_2$ or $(V_H\text{-}V_L)_2$), triabody (trivalent), tetrabody (tetravalent), minibody ($(scFv\text{-}C_H3)_2$), bispecific single-chain Fv (Bis-scFv), lgGdeltaCH2, scFv-Fc and $(scFv)_2$-Fc.

51. The antibody of of any of claims 1 to 4 or 26 to 30, wherein the antibody has at least 80%-85%, 85%-90%, 90%-95%, 96%, 97%, 98%, 99%, or more identity to heavy or light chain variable region sequence of SAM-6 antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

52. The antibody of any of claims 1 to 4 or 26 to 30, wherein the antibody further comprises a heterologous domain.

53. The antibody of claim 52, wherein the heterologous domain comprises a detectable label, tag or cytotoxic agent.

54. The antibody of claim 53, wherein the detectable label or tag comprises an enzyme; enzyme substrate; ligand; receptor; radionuclide; a T7-, His-, myc-, HA- or FLAG-tag; electron-dense reagent; energy transfer molecule; paramagnetic label; fluorophores; chromophore; chemi-luminescent agent; and bio-luminescent agent.

55. A kit comprising an antibody of any of claims 1 to 4 or 26 to 30 and instructions for detecting one or more of Grp78, apoB100, LDL, VLDL, oxidized LDL, deglycosylated Grp78 or deglycosylated LDL.

56. A kit comprising the antibody of any of claims 1 to 4 or 26 to 30 and instructions for treating undesirable cell proliferation or hyperproliferation.
57. The kit of claim 56, wherein the instructions are for treating a neoplasia, tumor or cancer.
58. The kit of claim 56, further comprising an anti-cell proliferative or immune enhancing treatment or therapeutic agent.
59. The kit of claim 56, further comprising an anti-neoplastic, anti-cancer or anti-tumor agent.
60. The kit of claim 56, wherein the instructions are on a label or packaging insert.
61. The kit of claim 56, further comprising an article of manufacture.
62. The kit of claim 61, wherein the article of manufacture is for delivering the antibody, anti-cell proliferative or immune enhancing treatment or therapy into a subject locally, regionally or systemically.
63. A pharmaceutical composition comprising the antibody of any of claims 1 to 4 or 26 to 30, and a pharmaceutically acceptable carrier or excipient.
64. A method for treating a cellular hyperproliferative disorder in a subject in need of treatment, comprising administering to the subject the antibody of any of claims 1 to 4 or 26 to 30 in an amount effective to treat the cellular hyperproliferative disorder in the subject.
65. The method of claim 64, wherein the cellular hyperproliferative disorder affects or is present at least in part in brain, head or neck, breast, esophagus, mouth, nasopharynx, nose or sinuses, stomach, duodenum, ileum, jejunum, lung, liver, pancreas, kidney, adrenal gland, thyroid, bladder, colon, rectum, prostate, uterus, cervix, ovary, bone marrow, lymph, blood, bone, testes, skin or muscle, or hematopoetic system.
66. The method of claim 64, wherein the cellular hyperproliferative disorder comprises a neoplasia, tumor or cancer.
67. The method of claim 66, wherein the neoplasia, tumor or cancer is metastatic or non-metastatic.
68. The method of claim 66, wherein the neoplasia, tumor or cancer affects or is at least in part present in breast, lung, thyroid, head and neck, nasopharynx, nose or sinuses, brain, spine, adrenal gland, thyroid, lymph, gastrointestinal tract, mouth, esophagus, stomach, duodenum, ileum, jejunum, small intestine, colon, rectum, genito-urinary tract, uterus, ovary, cervix, bladder, testicle, penis, prostate, kidney, pancreas, adrenal gland, liver, bone, bone marrow, lymph, blood, muscle or skin.
69. The method of claim 66, wherein the neoplasia, tumor or cancer is haematopoetic.

70. The method of claim 66, wherein the neoplasia, tumor or cancer comprises a sarcoma, carcinoma, adenocarcinoma, melanoma, myeloma, blastoma, glioma, lymphoma or leukemia.

71. The method of claim 66, wherein the neoplasia, tumor or cancer comprises a lung adenocarcinoma, lung carcinoma, diffuse or interstitial gastric carcinoma, colon adenocarcinoma, prostate adenocarcinoma, esophagus carcinoma, breast carcinoma, pancreas adenocarcinoma, ovarian adenocarcinoma, or uterine adenocarcinoma.

72. The method of claim 66, wherein the neoplasia, tumor or cancer comprises a stage I, II, III, IV or V metastatic or non-metastatic tumor or cancer.

73. The method of claim 66, wherein the neoplasia, tumor or cancer is progressively worsening.

74. The method of claim 66, wherein the neoplasia, tumor or cancer is in remission.

75. The method of claim 66, wherein the neoplasia, tumor or cancer is solid or liquid.

76. The method of claim 66, wherein the antibody is administered to the subject locally, regionally, or systemically.

77. The method of claims 65 or 66, wherein the treatment results in alleviating or ameliorating one or more adverse physical symptoms associated with the cellular hyperproliferative disorder, or the neoplasia, tumor or cancer.

78. The method of claim 66, wherein the treatment reduces or decreases neoplasia, tumor or cancer volume, inhibits or prevents an increase in neoplasia, tumor or cancer volume, inhibits neoplasia, tumor or cancer progression or worsening, stimulates neoplasia, tumor or cancer cell lysis or apoptosis, or inhibits, reduces or decreases neoplasia, tumor or cancer proliferation or metastasis.

79. The method of claims 65 or 66, wherein the treatment prolongs or extends lifespan of the subject.

80. The method of claims 65 or 66, wherein the treatment improves the quality of life of the subject.

81. The method of claims 65 or 66, wherein the subject is a candidate for, is undergoing, or has undergone an anti-neoplastic, anti-tumor, anti-cancer or immune-enhancing treatment or therapy.

82. The method of claims 65 or 66, further comprising administering to the subject an anti-cell proliferative or immune-enhancing treatment or therapy.

83. The method of claim 82, wherein the treatment or therapy comprises surgical resection, radiotherapy, radiation therapy, chemotherapy, immunotherapy, or hyperthermia.

84. The method of claim 82, wherein the anti-cell proliferative treatment or therapy comprises an alkylating agent, anti-metabolite, plant extract, plant alkaloid, nitrosourea, hormone, nucleoside or nucleotide analogue.

85. The method of claim 82, wherein the anti-cell proliferative treatment or therapy is selected from: cyclophosphamide, azathioprine, cyclosporin A, prednisolone, melphalan, chlorambucil, mechlorethamine, busulphan, methotrexate, 6-mercaptopurine, thioguanine, 5-fluorouracil, cytosine arabinoside, AZT, 5-azacytidine (5-AZC) and 5-azacytidine related compounds, bleomycin, actinomycin D, mithramycin, mitomycin C, carmustine, lomustine, semustine, streptozotocin, hydroxyurea, cisplatin, mitotane, procarbazine, dacarbazine, taxol, vinblastine, vincristine, doxorubicin and dibromomannitol.

86. The method of claim 82, wherein the immune enhancing treatment or therapy comprises a lymphocyte, plasma cell, macrophage, dendritic cell, NK cell or B-cell.

87. The method of claim 82, wherein the immune enhancing treatment or therapy comprises an antibody, a cell growth factor, a cell survival factor, a cell differentiative factor, a cytokine or a chemokine.

88. The method of claim 82, wherein the immune enhancing treatment or therapy is selected from: IL-2, IL-1α, IL-1β, IL-3, IL-6, IL-7, granulocyte-macrophage-colony stimulating factor (GMCSF), IFN-γ, IL-12, TNF-α, TNFβ, MIP-1α, MIP-1β, RANTES, SDF-1, MCP-1, MCP-2, MCP-3, MCP-4, eotaxin, eotaxin-2, I-309/TCA3, ATAC, HCC-1, HCC-2, HCC-3, LARC/MIP-3α, PARC, TARC, CKβ, CKβ6, CKβ7, CKβ8, CKβ9, CKβ11, CKβ12, C10, IL-8, GROα, GROβ, ENA-78, GCP-2, PBP/CTAPIIIβ-TG/NAP-2, Mig, PBSF/SDF-1, and lymphotactin.

89. The method of claim 82, wherein the antibody is administered prior to, substantially contemporaneously with or following administration of the anti-cell proliferative or immune-enhancing treatment or therapy.

90. The method of any of claims 64 to 89, wherein the subject is a mammal.

91. The method of claim 90, wherein the subject is a human.

92. The method of any of claims 64 to 89, wherein the subject is undergoing or has undergone treatment or therapy for a cellular hyperproliferative disorder.

93. A method for treating a disorder or disease associated with or caused by undesirable or excessive LDL or oxLDL levels, comprising administering to a subject an antibody of any of claims 1 to 4 or 26 to 30 in an amount effective to treat the disorder or disease associated with or caused by undesirable or excessive LDL or oxLDL levels in the subject.

94. A method of diagnosing a subject having or at increased risk of having a neoplasia, tumor or cancer, comprising:

a) providing a biological material or sample from the subject;

b) contacting the biological material or sample with the antibody of any of claims 1 to 4 or 26 to 30 for binding to a cell or antigen; and

c) assaying for binding of the antibody, wherein binding of the antibody to the cell or antigen diagnoses the subject as having or at increased risk of having a neoplasia, tumor or cancer.

95. The method of claim 94, wherein the biological material or sample is obtained from a human.

96. The method of claim 94, wherein the biological material or sample comprises a biopsy.

97. The method of claim 94, wherein the biological material or sample comprises a lung, pancreas, stomach, breast, esophageal, ovarian or uterine biopsy.

98. The method of claim 94, wherein the biological material or sample comprises serum, plasma, urine, saliva, nmenstruate, or feces.

99. The method of claim 94, wherein the antibody is distinct from SAM-6 antibody as represented by antibody produced by hybridoma deposited as DSM ACC2903, or a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18).

100. An isolated or purified antibody or a subsequence thereof that binds to an antigen present on both apoB100 protein and grp78 protein.

101. The antibody of claim 100, wherein the antibody competes for binding of SAM-6 antibody, as represented by antibody produced by hybridoma deposited as DSM ACC2903, or a light chain variable region sequence (SEQ ID NO:13) and a heavy chain variable region sequence (SEQ ID NO:15 or SEQ ID NO:18), for binding to apoB100 protein or grp78 protein.

102. The antibody or a subsequence thereof of any of claims 1 to 4, 26 to 30 and 100, wherein the antibody heavy chain variable region (V_H) alone can bind to glycosylated or deglycosylated Grp78, apoB100, glycosylated or deglycosylated LDL, glycosylated or deglycosylated VLDL or oxidized glycosylated or deglycosylated LDL.

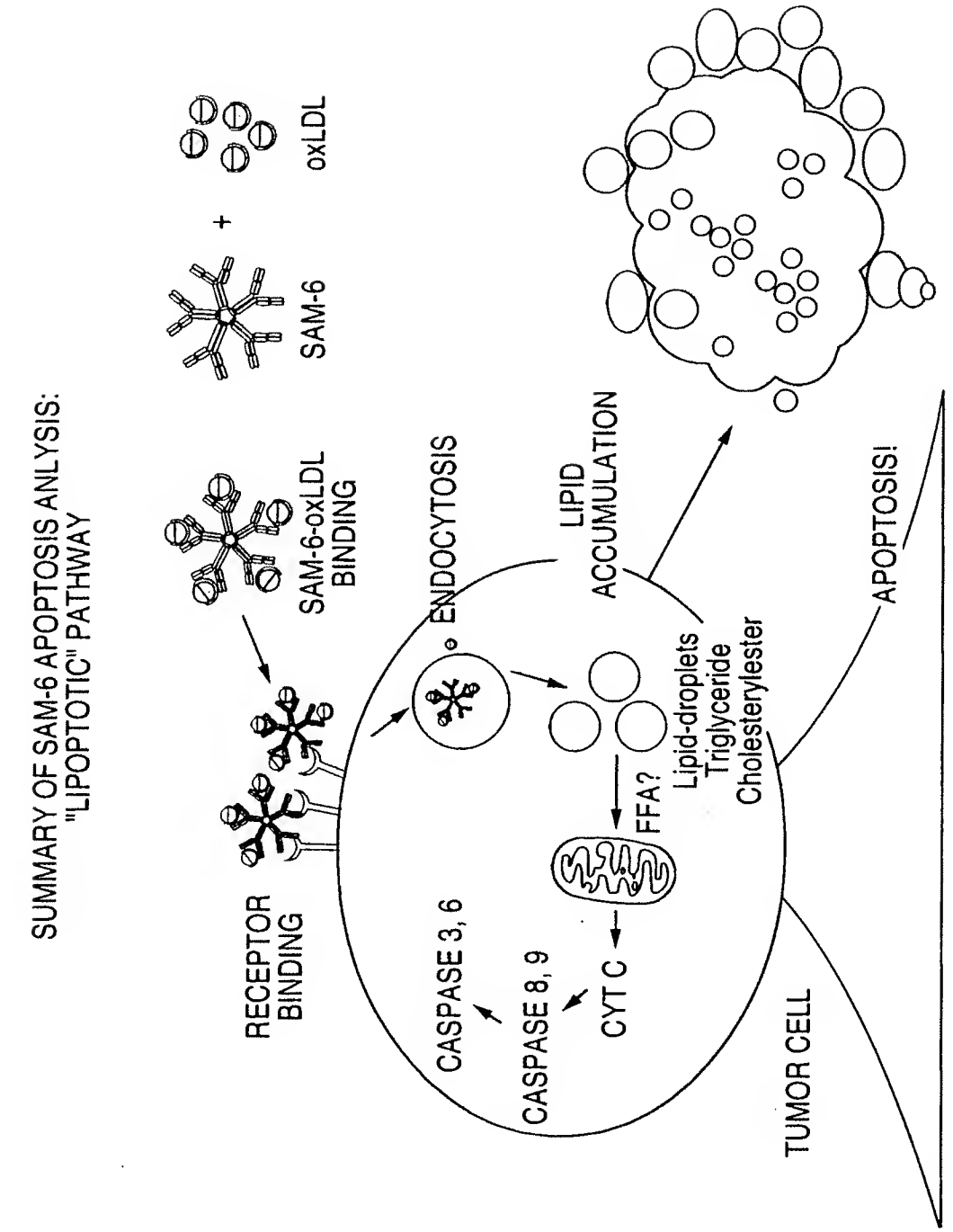

SUMMARY OF SAM-6 APOPTOSIS ANLYSIS:
"LIPOPTOTIC" PATHWAY
oxLDL
+
SAM-6
SAM-6-oxLDL
BINDING
RECEPTOR
BINDING
ENDOCYTOSIS
LIPID
ACCUMULATION
Lipid-droplets
Triglyceride
Cholesterylester
FFA?
CYT C
CASPASE 8, 9
CASPASE 3, 6
TUMOR CELL
APOPTOSIS!

FIG. 1

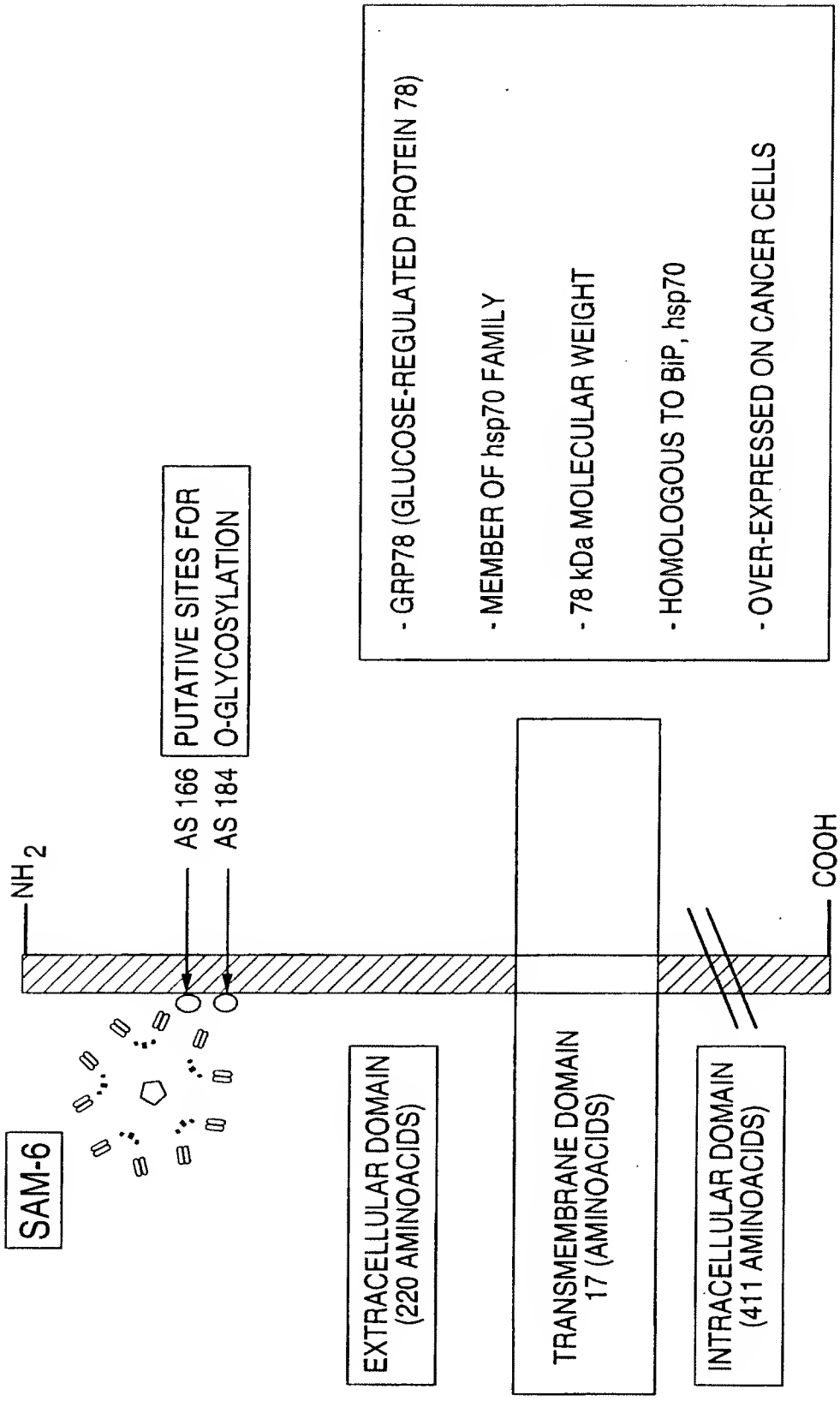

SAM-6 TARGET
PUTATIVE SITES FOR O-GLYCOSYLATION
AS 166
AS 184
NH2
COOH
SAM-6
EXTRACELLULAR DOMAIN (220 AMINOACIDS)
TRANSMEMBRANE DOMAIN 17 (AMINOACIDS)
INTRACELLULAR DOMAIN (411 AMINOACIDS)
- GRP78 (GLUCOSE-REGULATED PROTEIN 78)
- MEMBER OF hsp70 FAMILY
- 78 kDa MOLECULAR WEIGHT
- HOMOLOGOUS TO BiP, hsp70
- OVER-EXPRESSED ON CANCER CELLS

FIG. 2

Representative western blot of SAM-6 and unrelated IgM control on membrane extracts

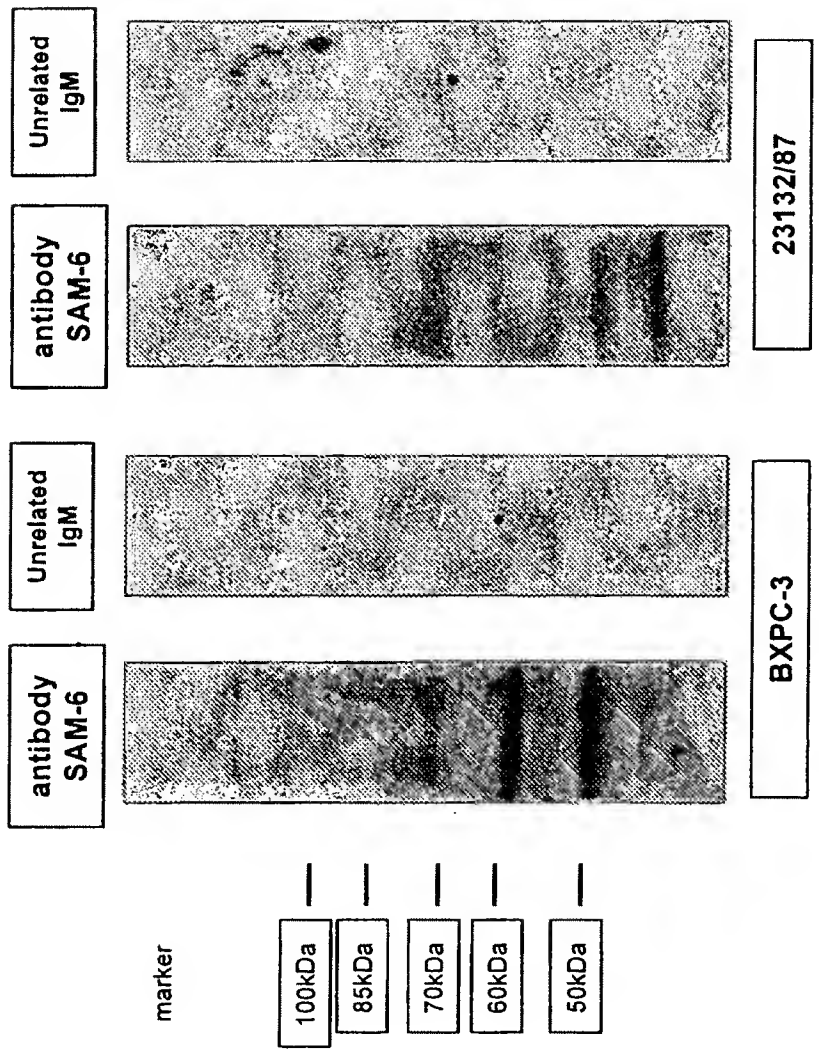


Figure 3

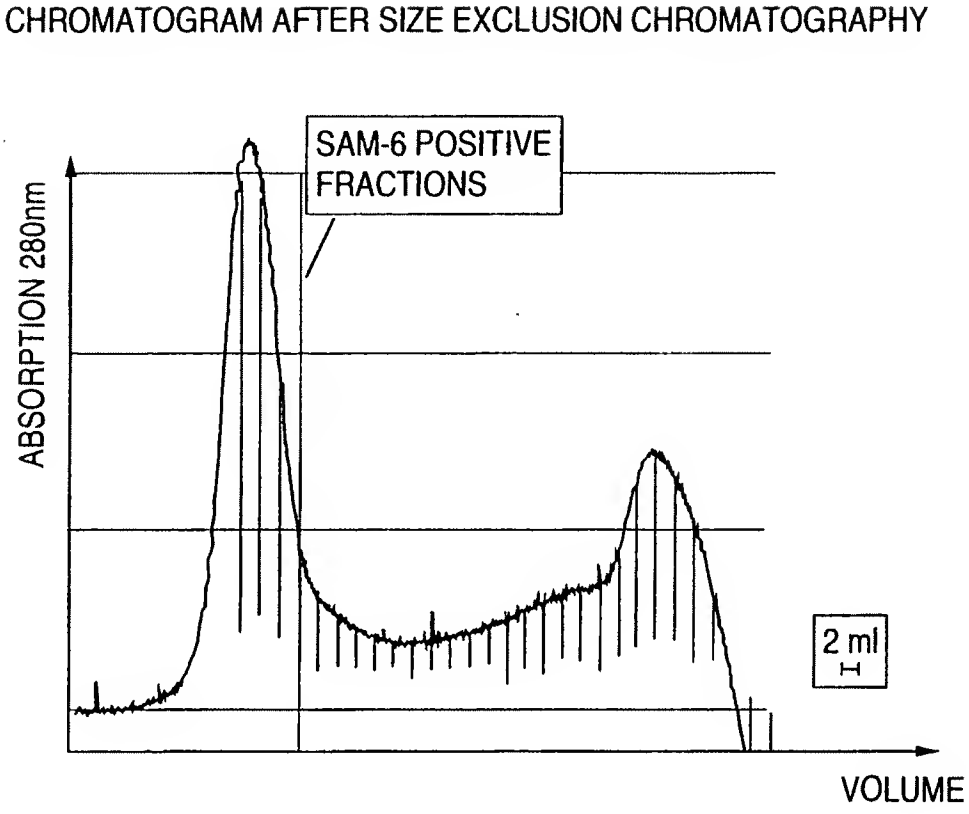

CHROMATOGRAM AFTER SIZE EXCLUSION CHROMATOGRAPHY
SAM-6 POSITIVE FRACTIONS
ABSORPTION 280nm
2 ml
VOLUME

FIG. 4

Western blot analysis of SAM-6 positive fractions after size exclusion chromatography

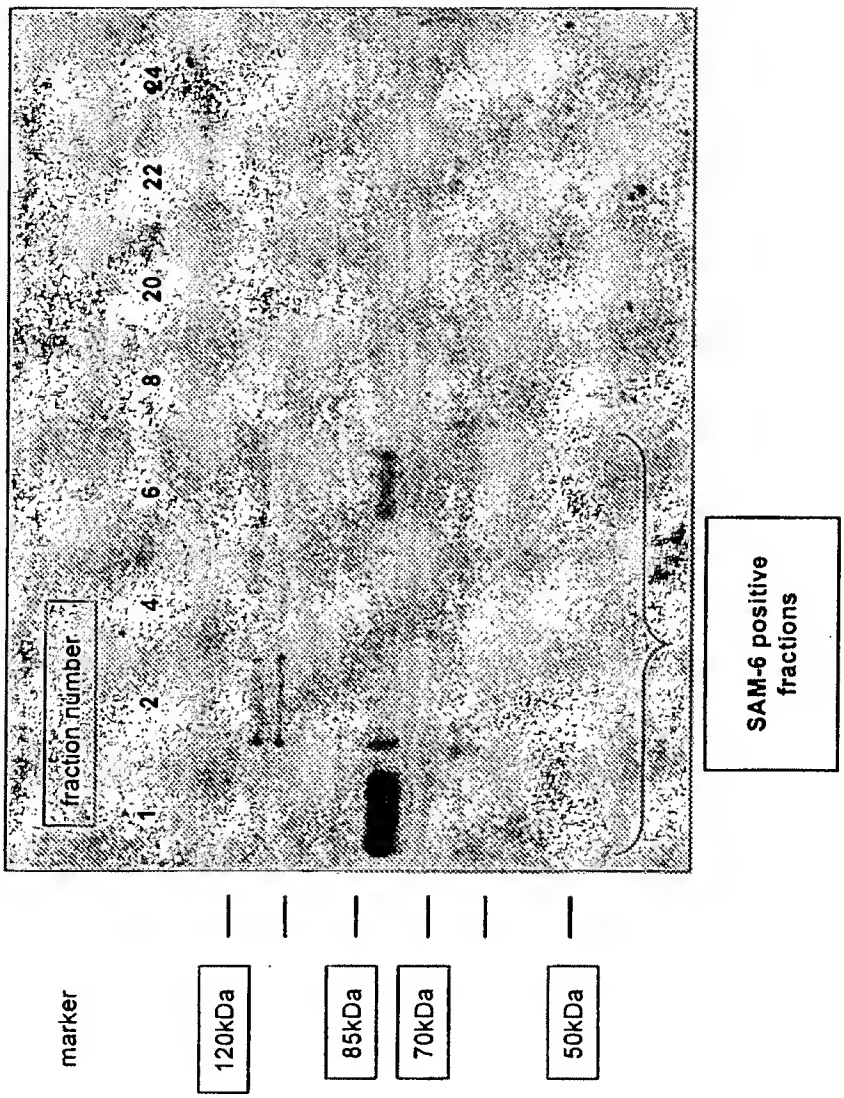


Figure 5

Coomassie staining of SDS-Page gel after size exclusion chromatography

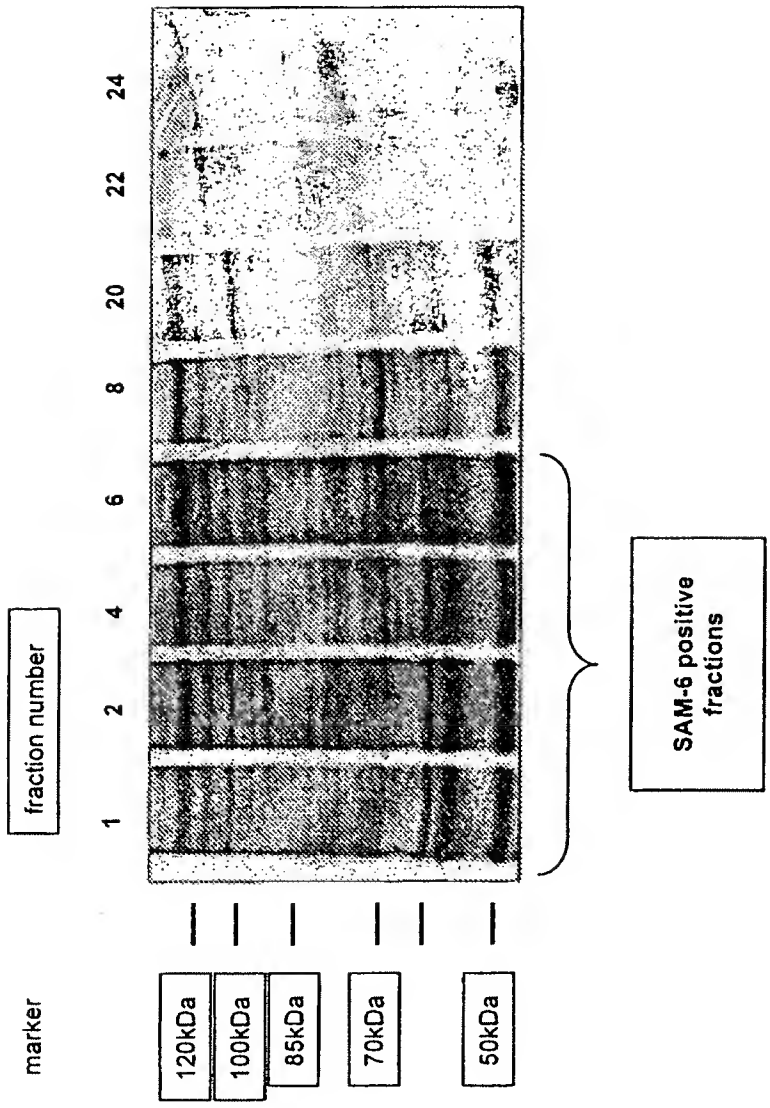


Figure 6

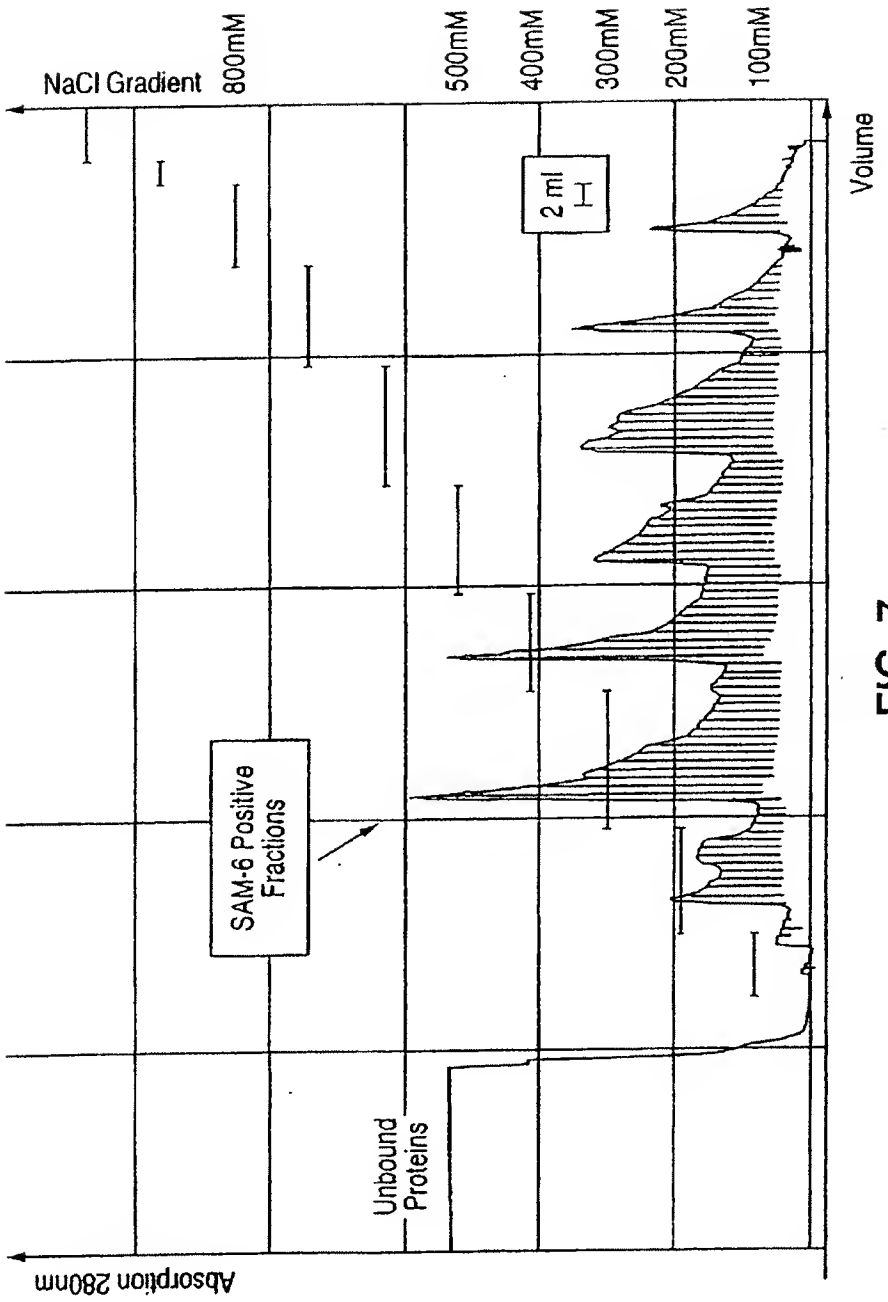

NaCl Gradient
800mM
500mM
400mM
300mM
200mM
100mM
2 ml
Volume
SAM-6 Positive Fractions
Unbound Proteins
Absorption 280nm

FIG. 7

Western blot analysis of SAM-6 positive fractions after ion exchange chromatography

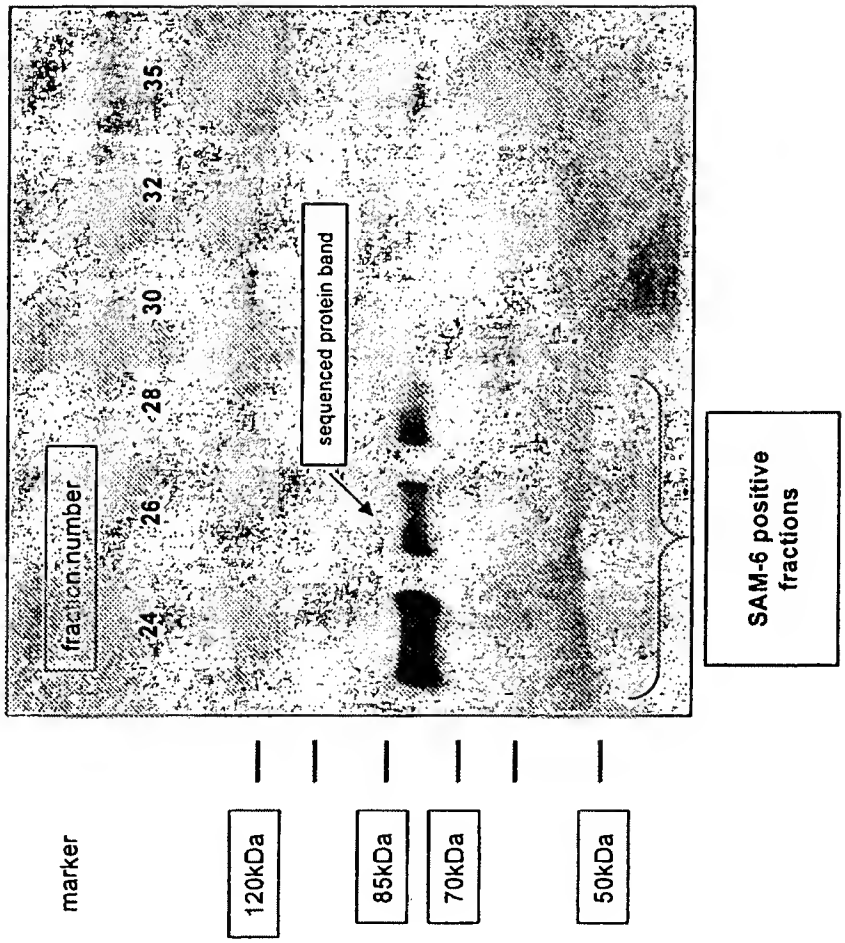


Figure 8

Coomassie Blue Staining after ion exchange chromatography

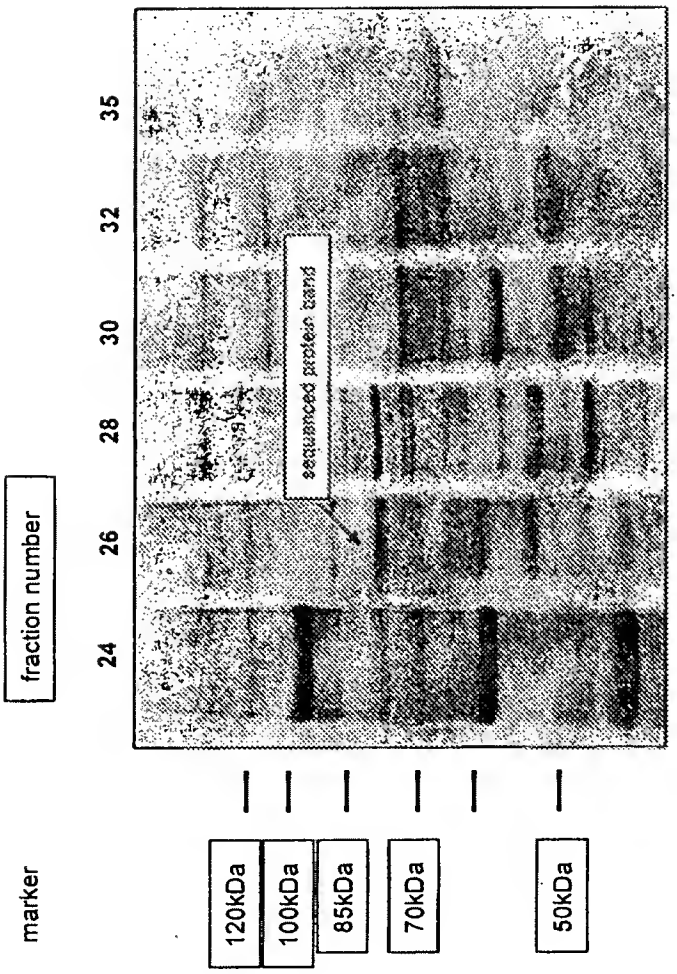


Figure 9

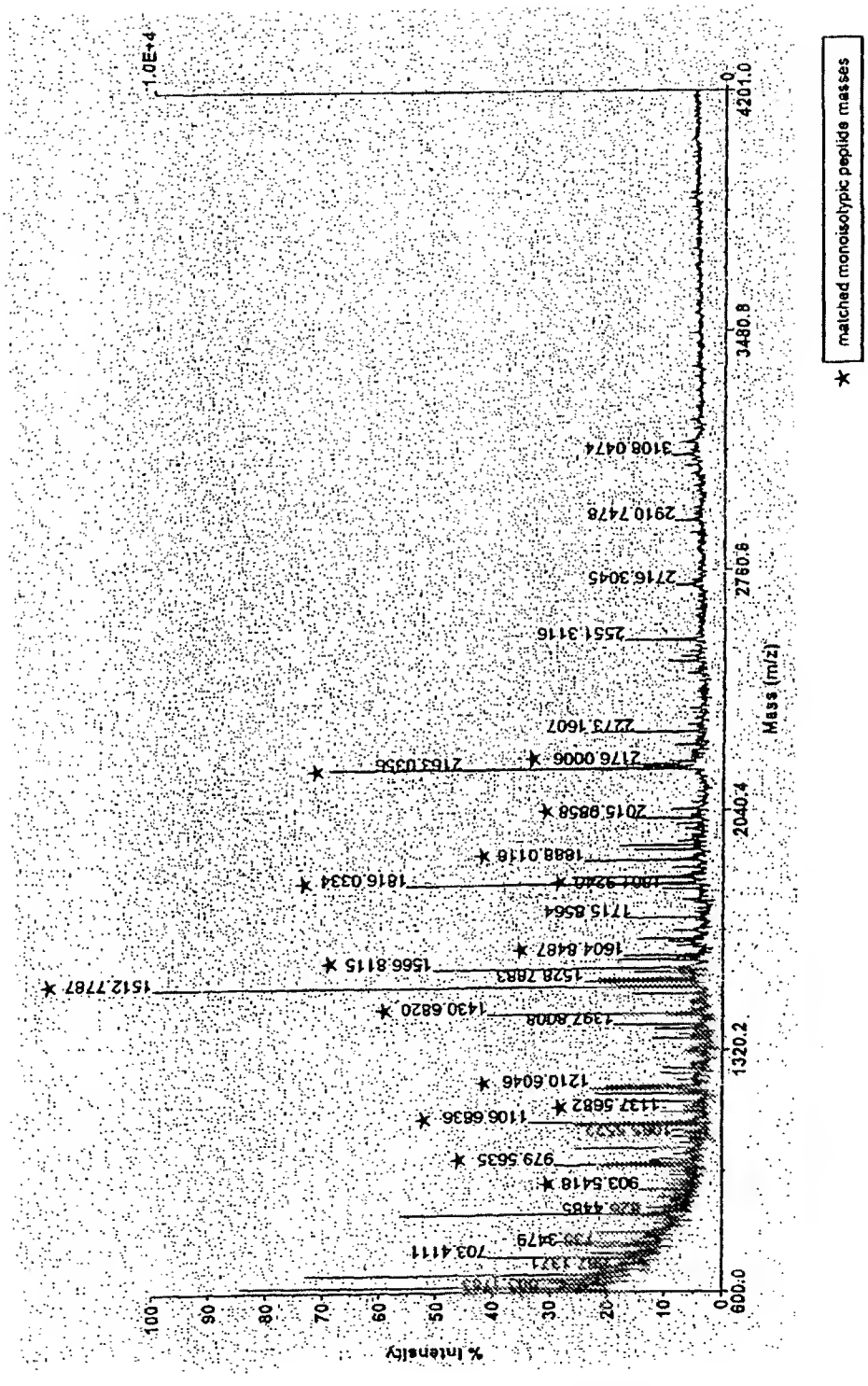


Figure 10: Peptide mass mapp of isolated 80kDa protein obtained by MALDI mass spectrometry analysis

Alignment of experimentally derived peptide sequences assigned to human GRP78 and the database protein sequence of human GRP78/BiP [NP_005338], SEQ ID NO:1

ER translocation/ cleavage site

1 N mklslvaaml lllsaaraee edkkedvgtv vgidlgttys cvgvfk**ngrv eiiandqgnr**

61 **itpsyvaftp egerligdaa knqltsnpen tvfdakrlig rtwndpsvqq dikflpfkvv**

121 **ekktkpyiqv digggqtktf apeeisamvl tk**mketaeay lgk**kvthavv tvpayfndaq**

Transmembrane domain

181 **rqatkdagti aglnvmr**iin eptaaaiayg ldkregekni lvfdlgggtf dvslltidng

241 vfevvatngd thlggedfdq r**vmehfik**ly kkktgkdvrk dnravqklrr evekakrals

301 sqhqarieie sfyegedfse tltr**akfeel nmdlfrstmk pvqkvledsd lk**ksdideiv

361 lvggstripk iqqlvk**effn gkepsr**ginp deavaygaav qagvlsgdqd tgdlvlldvc

421 pltlgietvg gvmtkliprn tvvptkksqi fstasdnqpt vtik**vyeger pltkdnhllg**

481 **tfdltgippa pr**gvpqievt feidvngilr vtaedkgtgn knkititndq nr**ltpeeier**

541 mvndaekfae edkklker**id trnelesyay slk**nqigdke klggklssed ketmekavee

ATPase activity

601 kiewleshqd adiedfkakk keleeivqpi isk**lygsagp pptgeedtae** kdel C

bolded - matched peptides outline - possible extracellular O-glycosylation sites signal region

FIG. 11

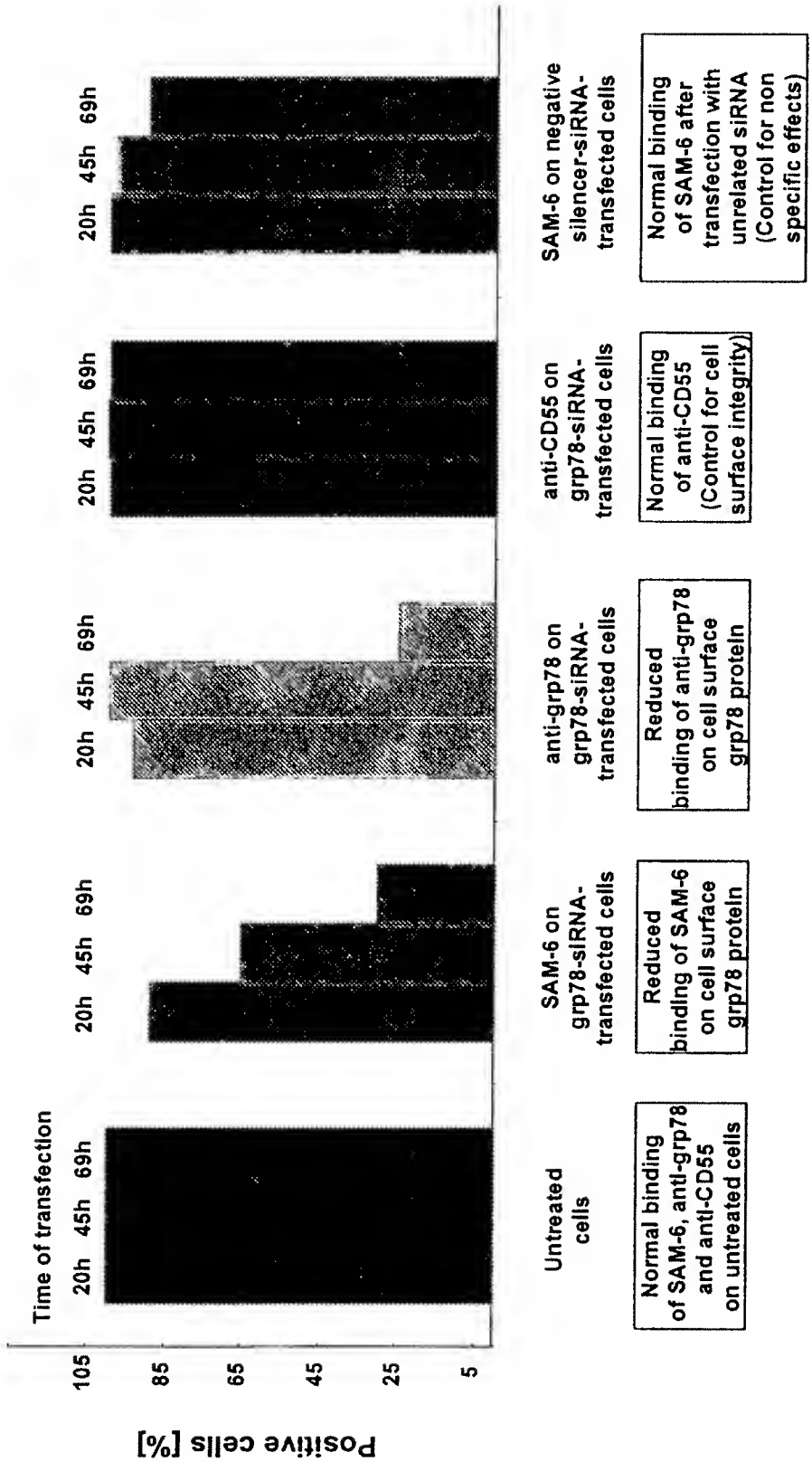

FACS analysis of SAM-6 binding on grp78-siRNA-transfected BXPC-3 cells
Positive cells [%]
105
85
65
45
25
5
Time of transfection
20h 45h 69h
Untreated cells
Normal binding of SAM-6, anti-grp78 and anti-CD55 on untreated cells
SAM-6 on grp78-siRNA-transfected cells
Reduced binding of SAM-6 on cell surface grp78 protein
anti-grp78 on grp78-siRNA-transfected cells
Reduced binding of anti-grp78 on cell surface grp78 protein
anti-CD55 on grp78-siRNA-transfected cells
Normal binding of anti-CD55 (Control for cell surface integrity)
SAM-6 on negative silencer-siRNA-transfected cells
Normal binding of SAM-6 after transfection with unrelated siRNA (Control for non specific effects)

Figure 12

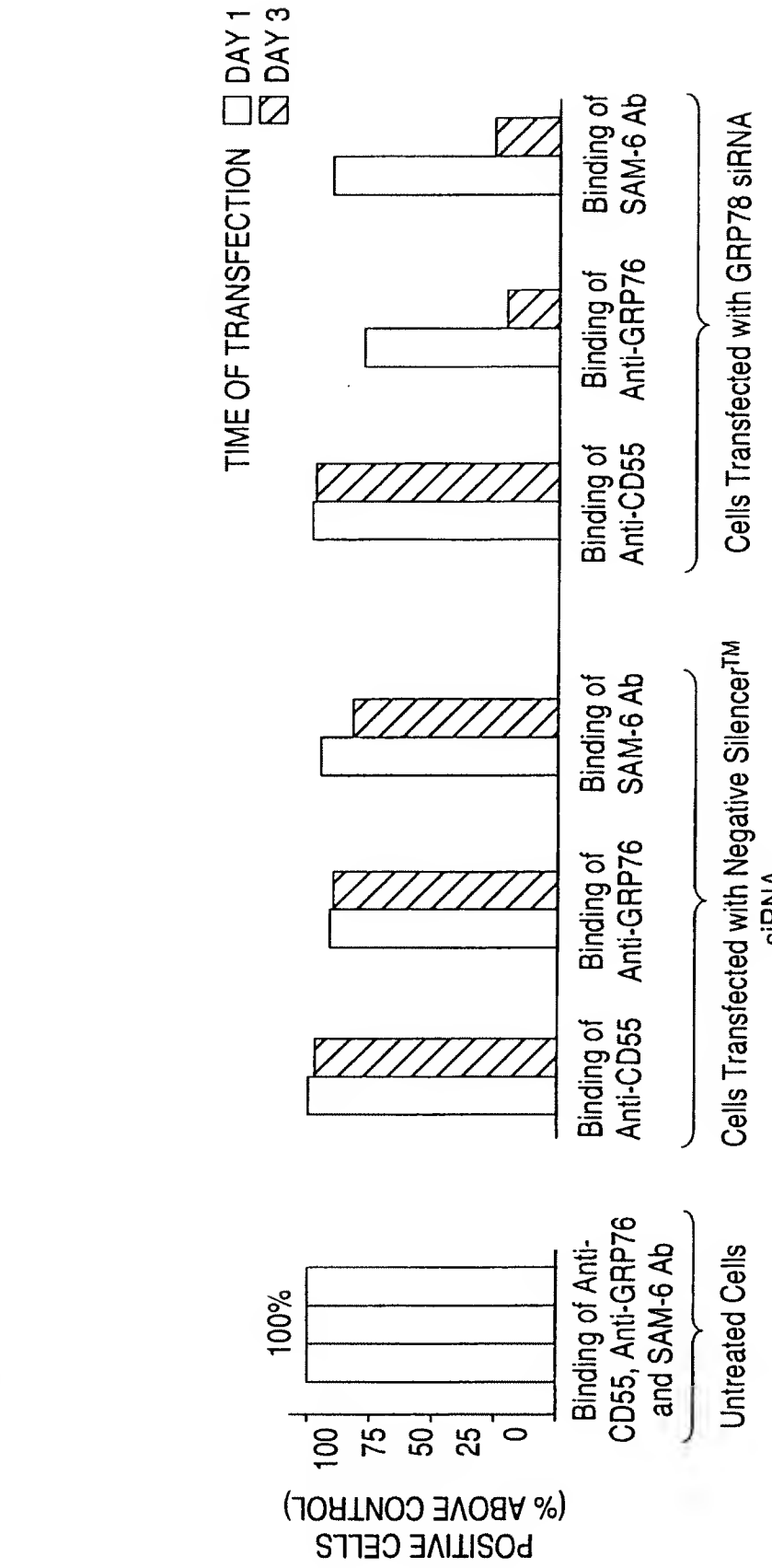

TIME OF TRANSFECTION
DAY 1
DAY 3
POSITIVE CELLS
(% ABOVE CONTROL)
100%
100
75
50
25
0
Binding of Anti-CD55, Anti-GRP76 and SAM-6 Ab
Untreated Cells
Binding of Anti-CD55
Binding of Anti-GRP76
Binding of SAM-6 Ab
Cells Transfected with Negative Silencer™ siRNA
Binding of Anti-CD55
Binding of Anti-GRP76
Binding of SAM-6 Ab
Cells Transfected with GRP78 siRNA

FIG. 13

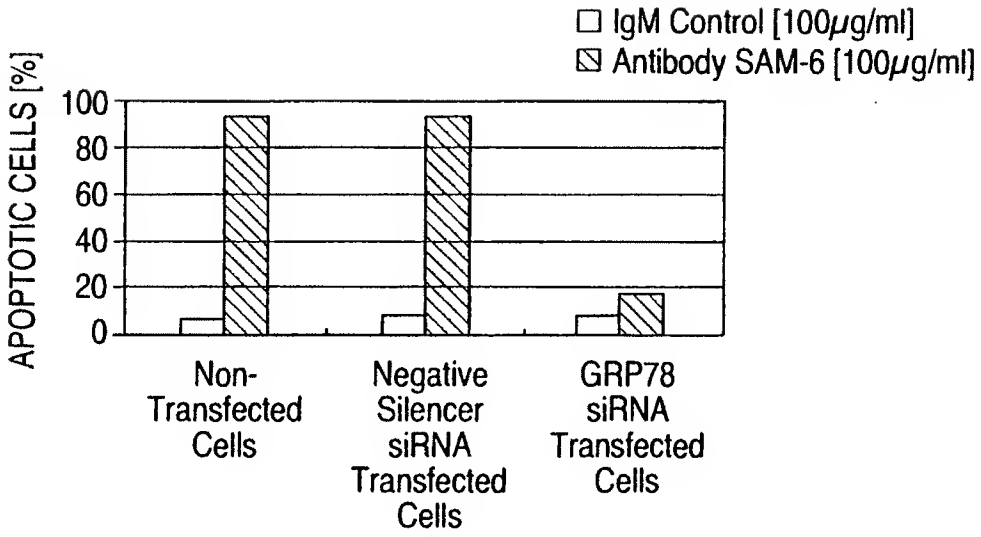
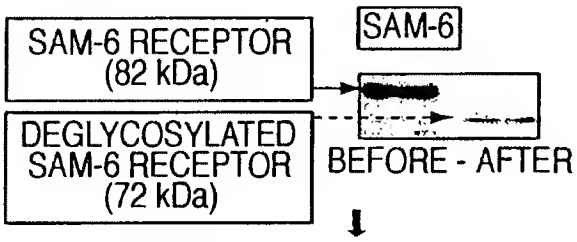

IgM Control [100μg/ml]
Antibody SAM-6 [100μg/ml]
APOPTOTIC CELLS [%]
100
80
60
40
20
0
Non-Transfected Cells
Negative Silencer siRNA Transfected Cells
GRP78 siRNA Transfected Cells

FIG. 14

WESTERN BLOT ANALYSIS
OF SAM-6 ANTIBODY ON MEMBRANE EXTRACTS OF PANCREAS
CARCINOMA CELL LINE BXPC-3
AFTER DEGLYCOSYLATION



DECREASE OF MOLECULAR WEIGHT AND BINDING INTENSITY
AFTER
N - AND O - DEGLYCOSYLATION
UNDER REDUCED CONDITIONS

FIG. 15

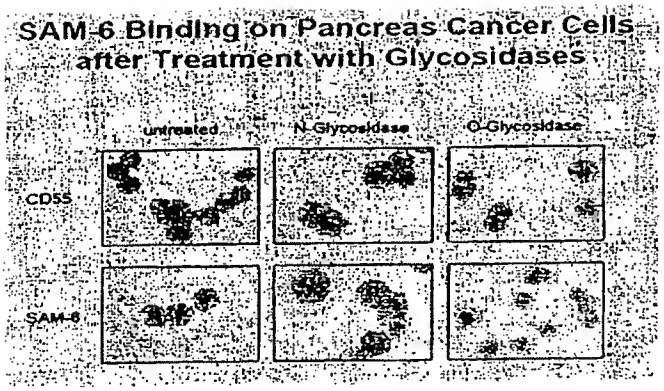


Figure 16: SAM-6 binding on pancreas cancer cells after treatment with glycosidases

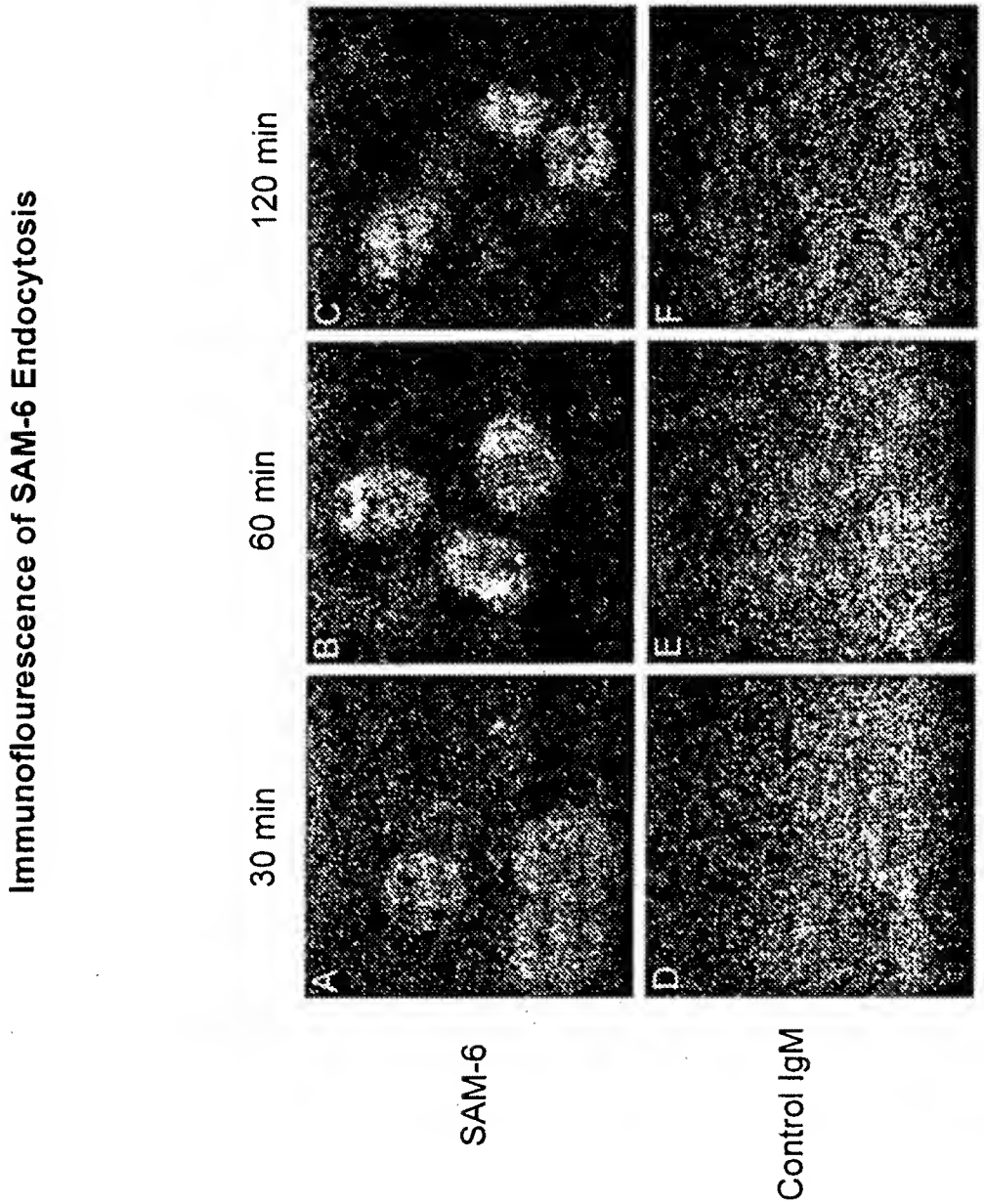

Immunoflourescence of SAM-6 Endocytosis
30 min
60 min
120 min
A
B
C
D
E
F
SAM-6
Control IgM

Figure 17

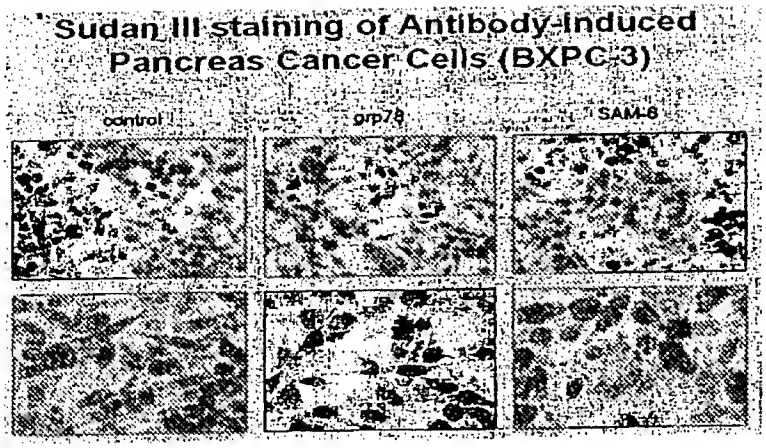


Figure 18: Sudan III staining of antibody-induced pancreas cancer cells (BXPC-3)

Analysis of SAM-6 induced apoptosis by measurement of cytochrom c release

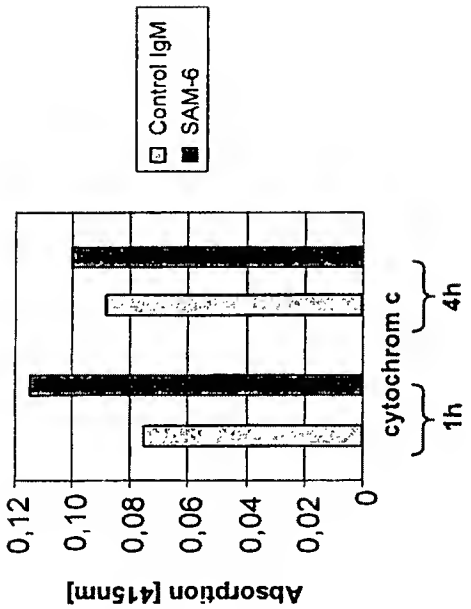


Figure 19

Analysis of SAM-6 induced apoptosis by measurement of activation of caspases -8, -9, -3 and -6

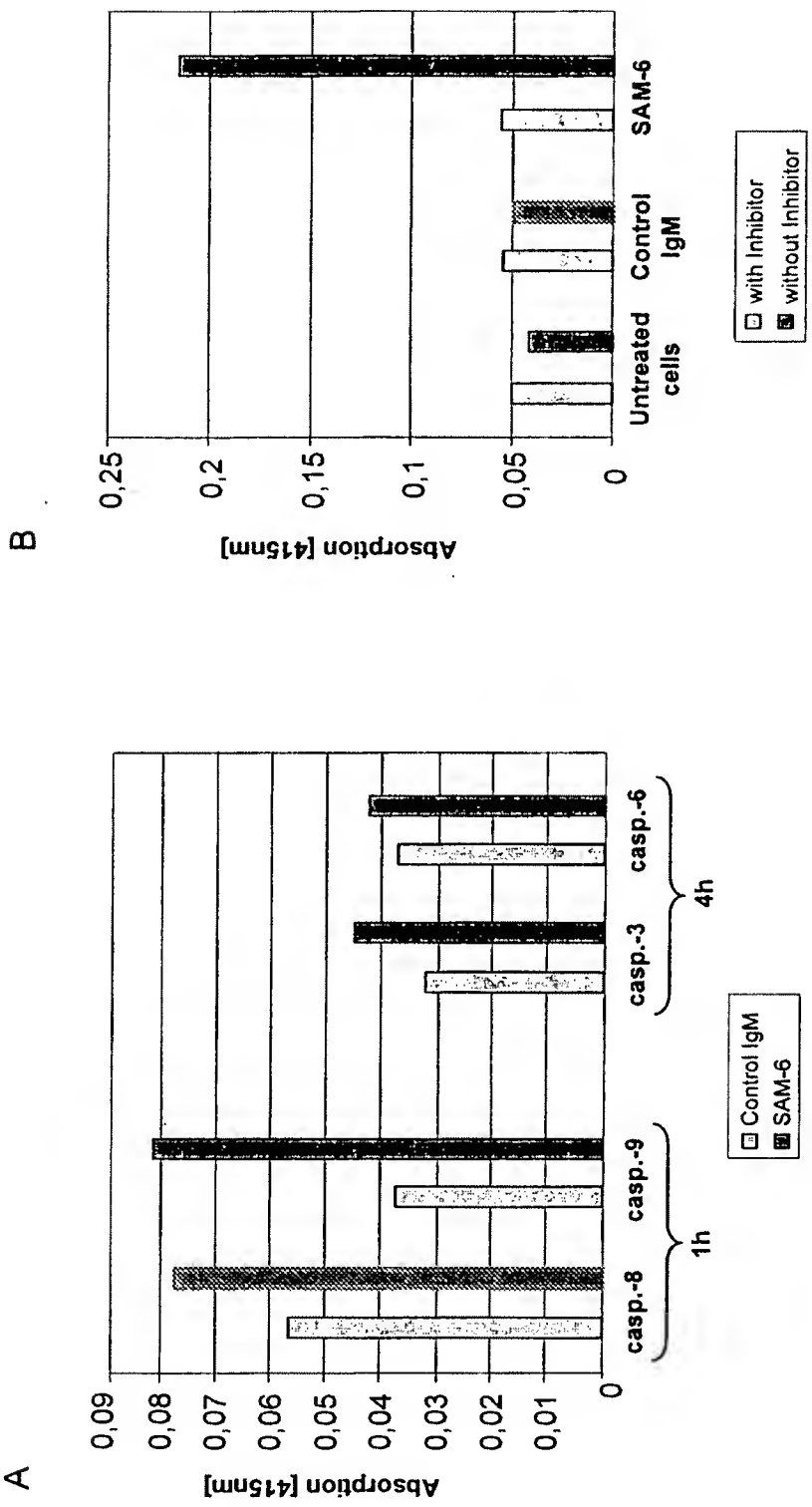


Figure 20

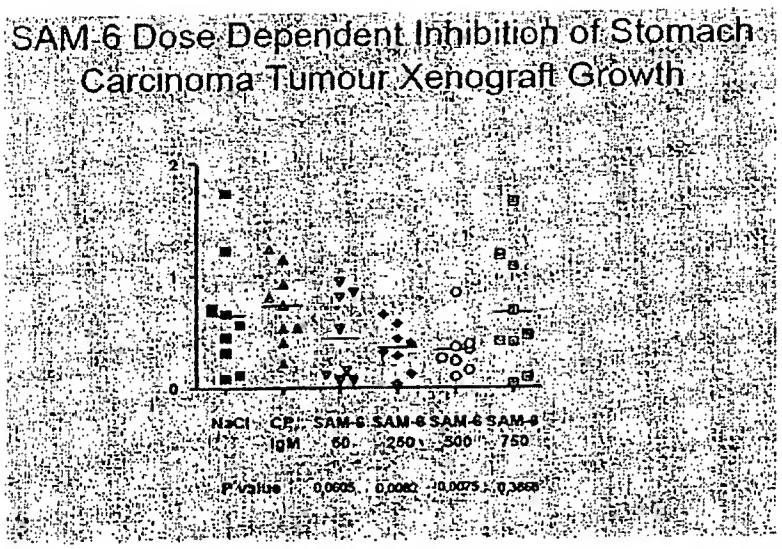


Figure 21: SAM-6 Dose dependent inhibition of stomach carcinoma tumour xenograft growth

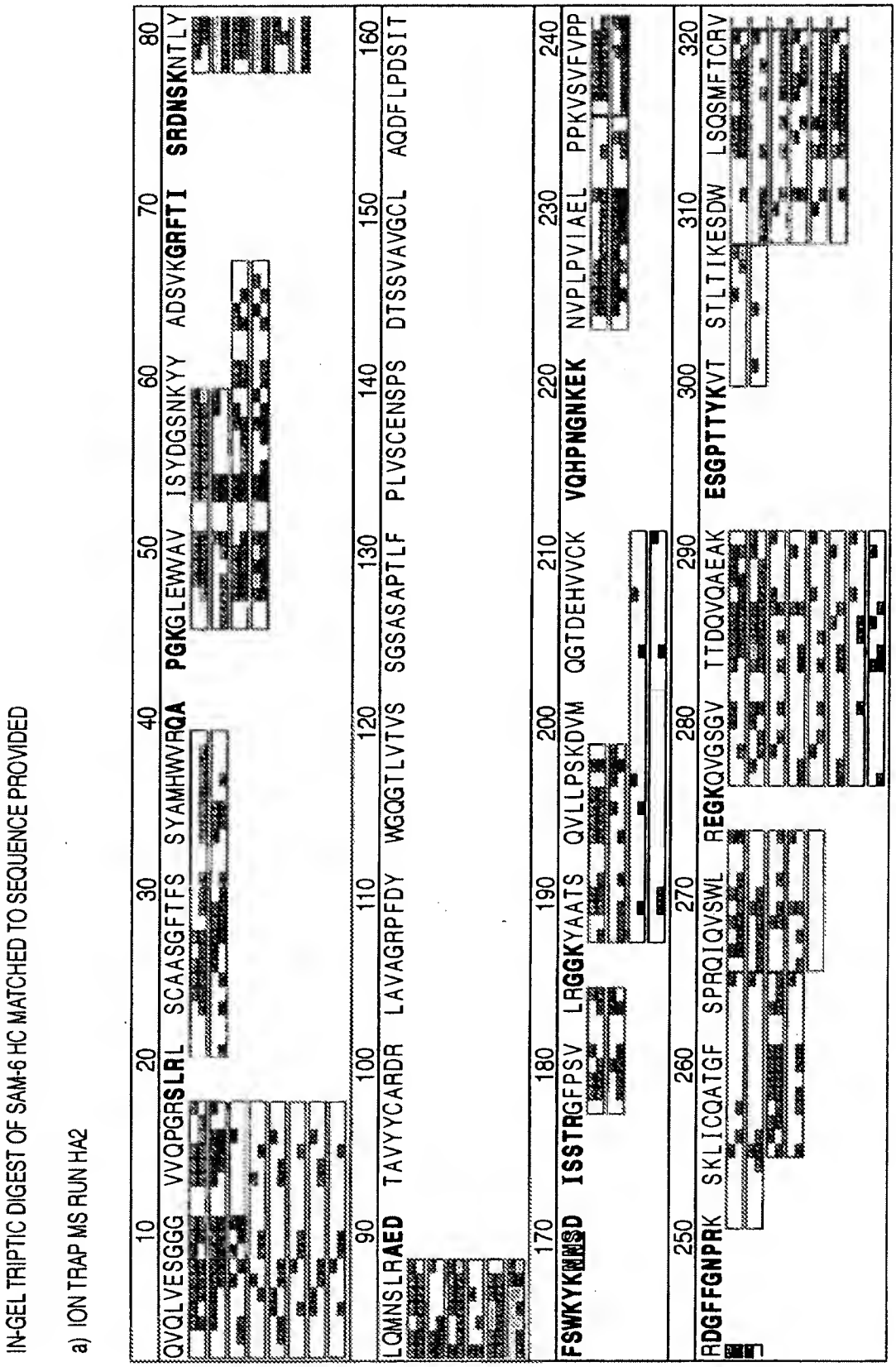

IN-GEL TRIPTIC DIGEST OF SAM-6 HC MATCHED TO SEQUENCE PROVIDED
a) ION TRAP MS RUN HA2
10 20 30 40 50 60 70 80
QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI SRDNSKNTLY
90 100 110 120 130 140 150 160
LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS SGSASAPTLF PLVSCENSPS DTSSVAVGCL AQDFLPDSIT
170 180 190 200 210 220 230 240
FSWKYKNNSD ISSTRGFPSV LRGGKYAATS QVLLPSKDVM QGTDEHVVCK VQHPNGNKEK NVPLPVIAEL PPKVSVFVPP
250 260 270 280 290 300 310 320
RDGFFGNPRK SKLICQATGF SPRQIQVSWL REGKQVGSGV TTDQVQAEAK ESGPTTYKVT STLTIKESDW LSQSMFTCRV

FIG. 22A(1)

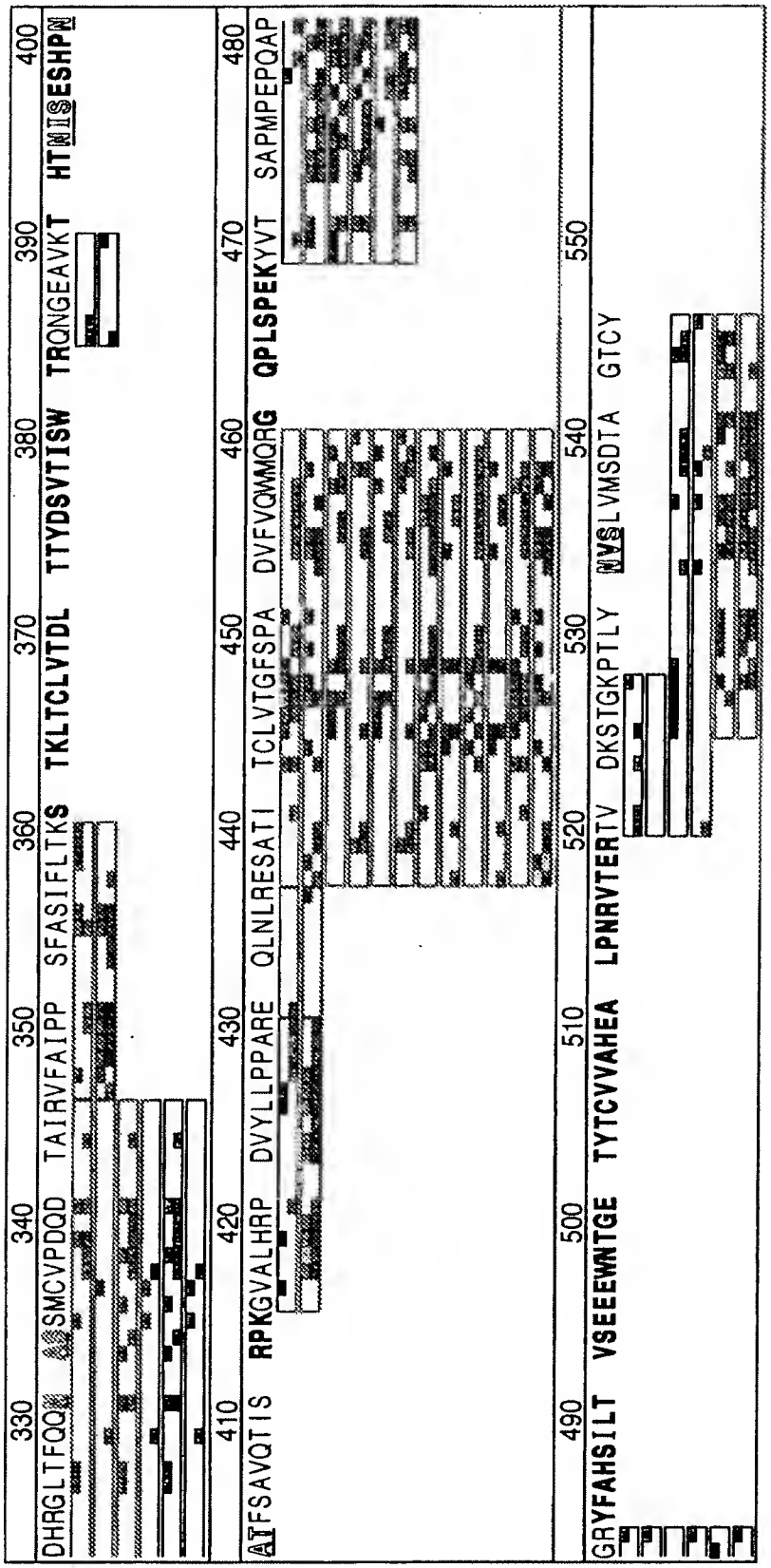

330 340 350 360 370 380 390 400
DHRGLTFQQ SMCVPDQD TAIRVFAIPP SFASIFLTKS TKLTCLVTDL TTYDSVTISW TRQNGEAVKT HTNISESHPN
410 420 430 440 450 460 470 480
ATFSAVQTIS RPKGVALHRP DVYLLPPARE QLNLRESATI TCLVTGFSPA DVFVQWMQRG QPLSPEKYVT SAPMPEPQAP
490 500 510 520 530 540 550
GRYFAHSILT VSEEEWNTGE TYTCVVAHEA LPNRVTERTV DKSTGKPTLY NVSLVMSDTA GTCY

FIG. 22A(2)

b) LC-MALDI MS RUN OF IN-GEL TRYPTIC DIGEST OF SAM-6 HC

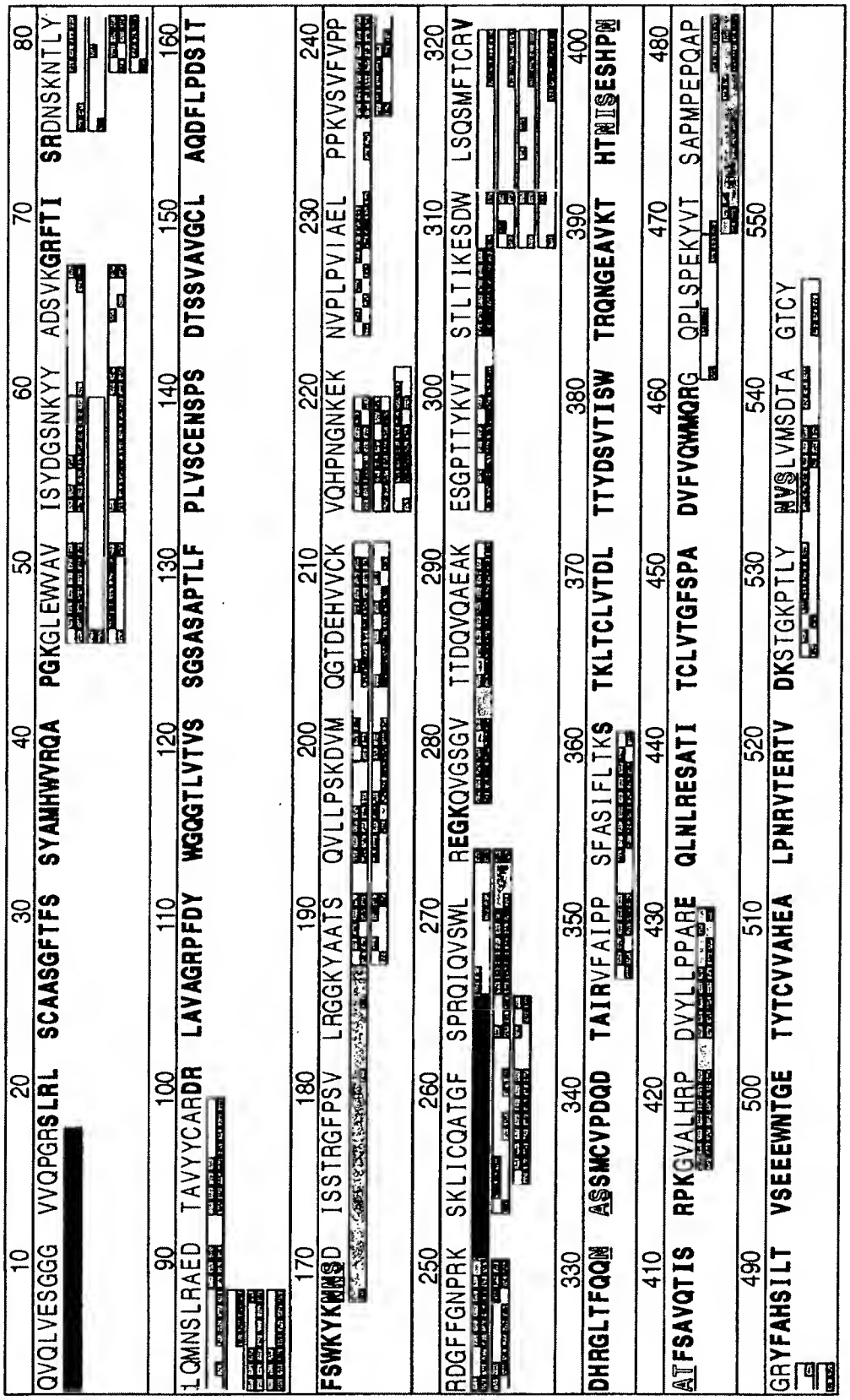


FIG. 22B

SEQUENCE COVERAGE OF SAM-6 LIGHT CHAIN

a) LC-ESI-ION TRAP MS

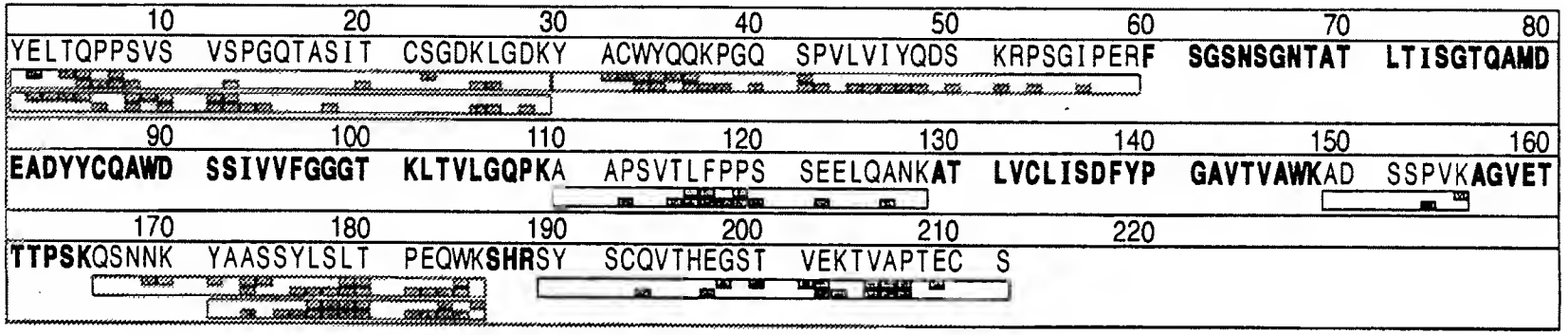


FIG. 23A

b) LC-MALDI MS

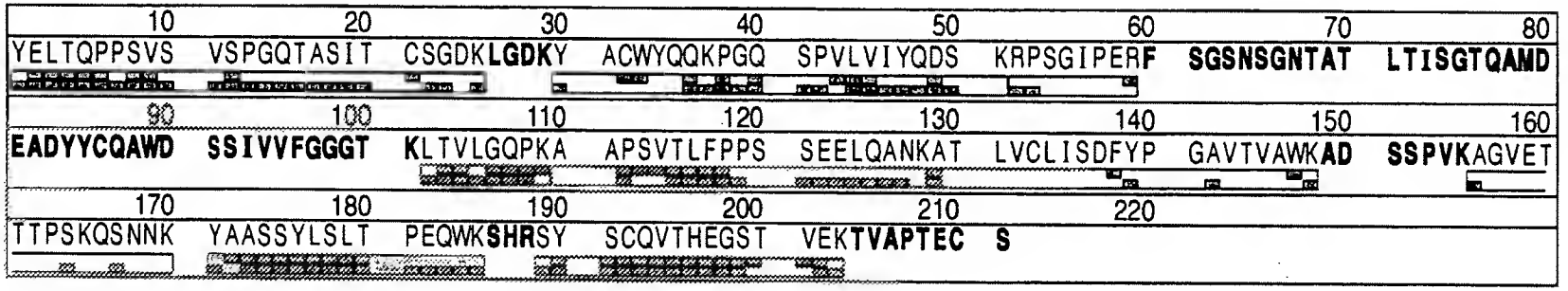


FIG. 23B

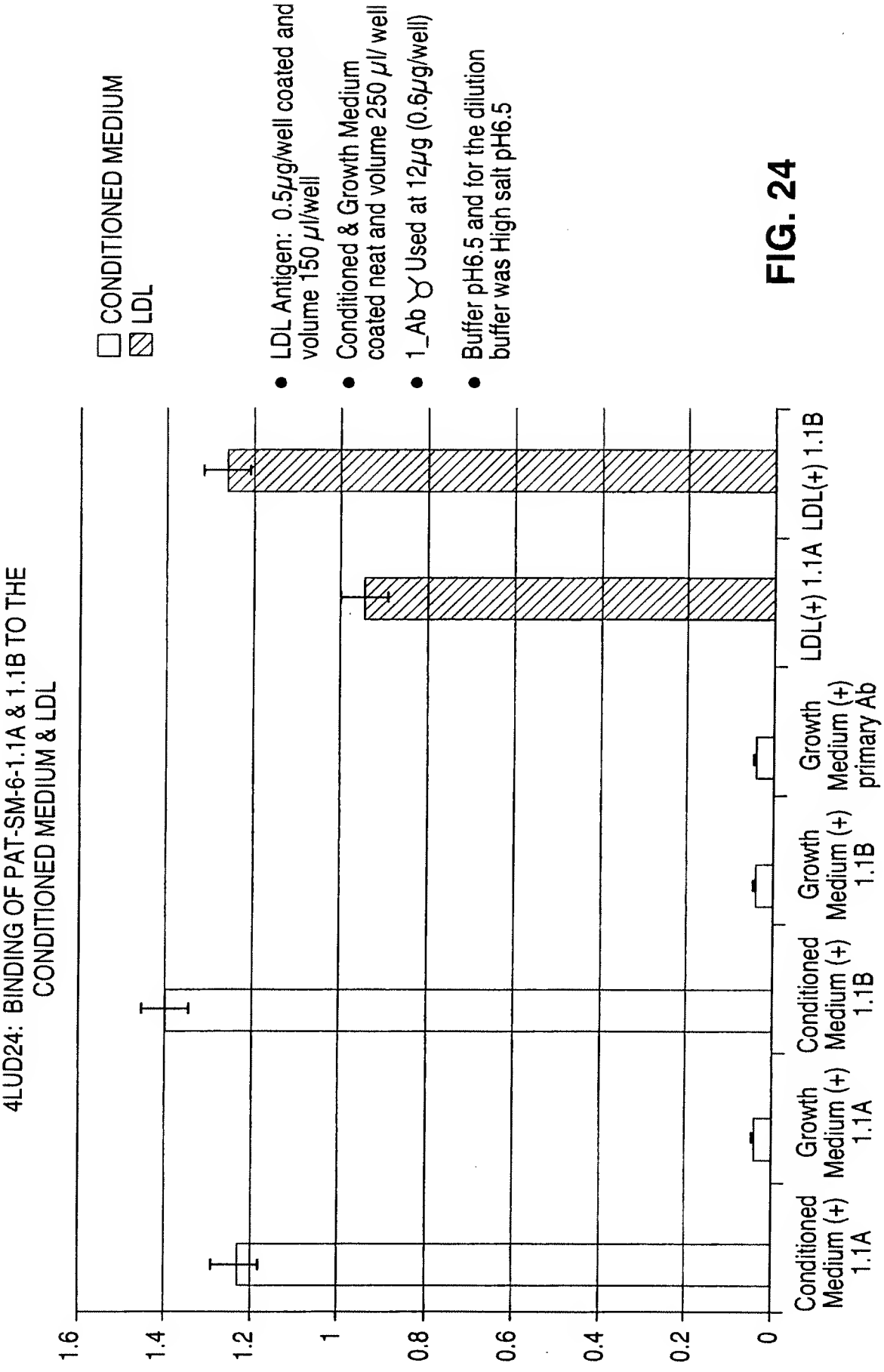
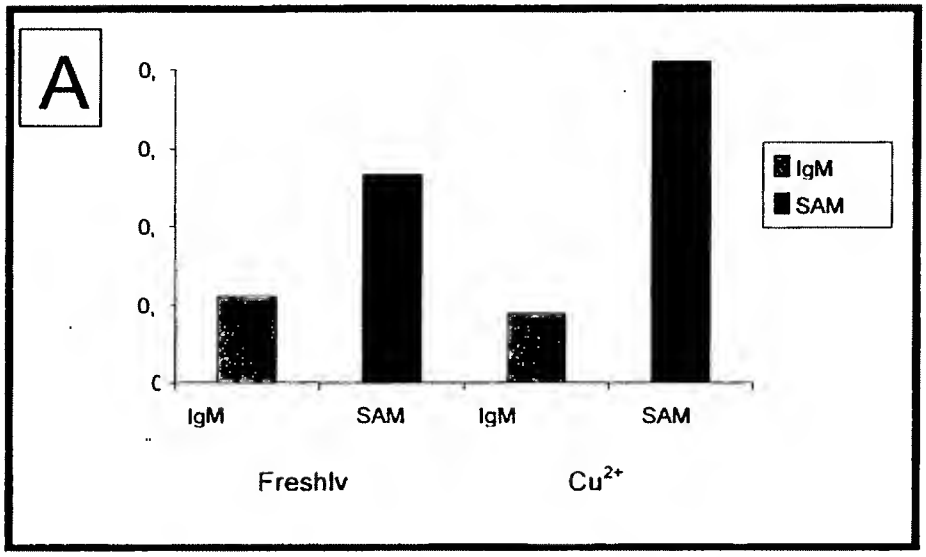

4LUD24: BINDING OF PAT-SM-6-1.1A & 1.1B TO THE CONDITIONED MEDIUM & LDL
CONDITIONED MEDIUM
LDL
LDL Antigen: 0.5µg/well coated and volume 150 µl/well
Conditioned & Growth Medium coated neat and volume 250 µl/ well
1_Ab Used at 12µg (0.6µg/well)
Buffer pH6.5 and for the dilution buffer was High salt pH6.5
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Conditioned Medium (+) 1.1A
Growth Medium (+) 1.1A
Conditioned Medium (+) 1.1B
Growth Medium (+) 1.1B
Growth Medium (+) primary Ab
LDL(+) 1.1A
LDL(+) 1.1B

FIG. 24

Figure 25

Functional cell binding and cell death ELISA



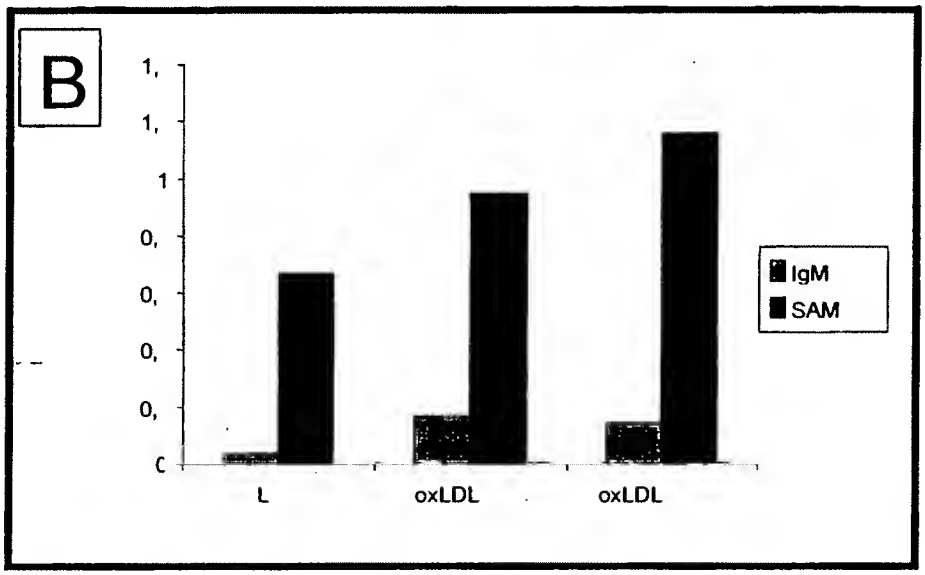
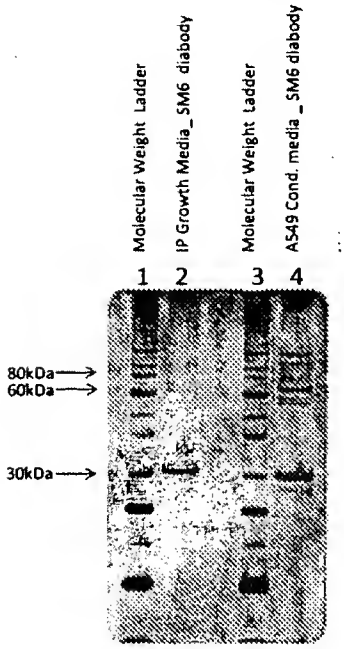


Figure 26

Immunoprecipitation: SM6 diabody binding to Conditioned media from A549 cells (05.02.09)

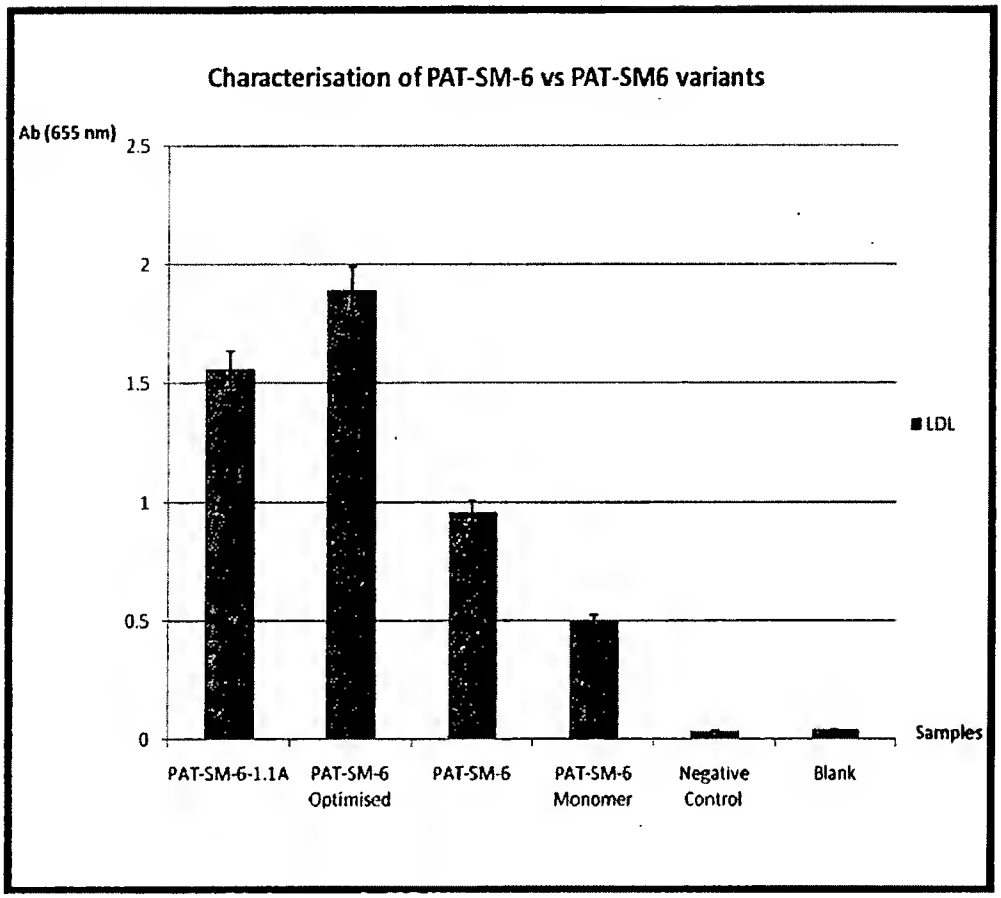


10% Bis-Tris SDS-PAGE gel stained with silver stain
Lane 1: molecular weight marker (30kDa band highlighted)
Lane 2: negative control- growth media (no contact with cells), plus SM6 diabody
Lane 3: molecular weight marker (260, 160, 110, 80, 60, 50, 40, 30, 20, 15, 10, 3,5kDa)
Lane 4: conditioned media from A549 cells (cells removed), plus SM6 diabody

Lane 2: shows us the size of the SM6 diabody (30kDa), and that there are no other proteins precipitated from the growth media.
Lane 4 : shows us that there are several proteins in the A549 conditioned media that bind to SM6. We will identify these by Mass Spectrometry.

Figure 27

LDL ELISA of variants



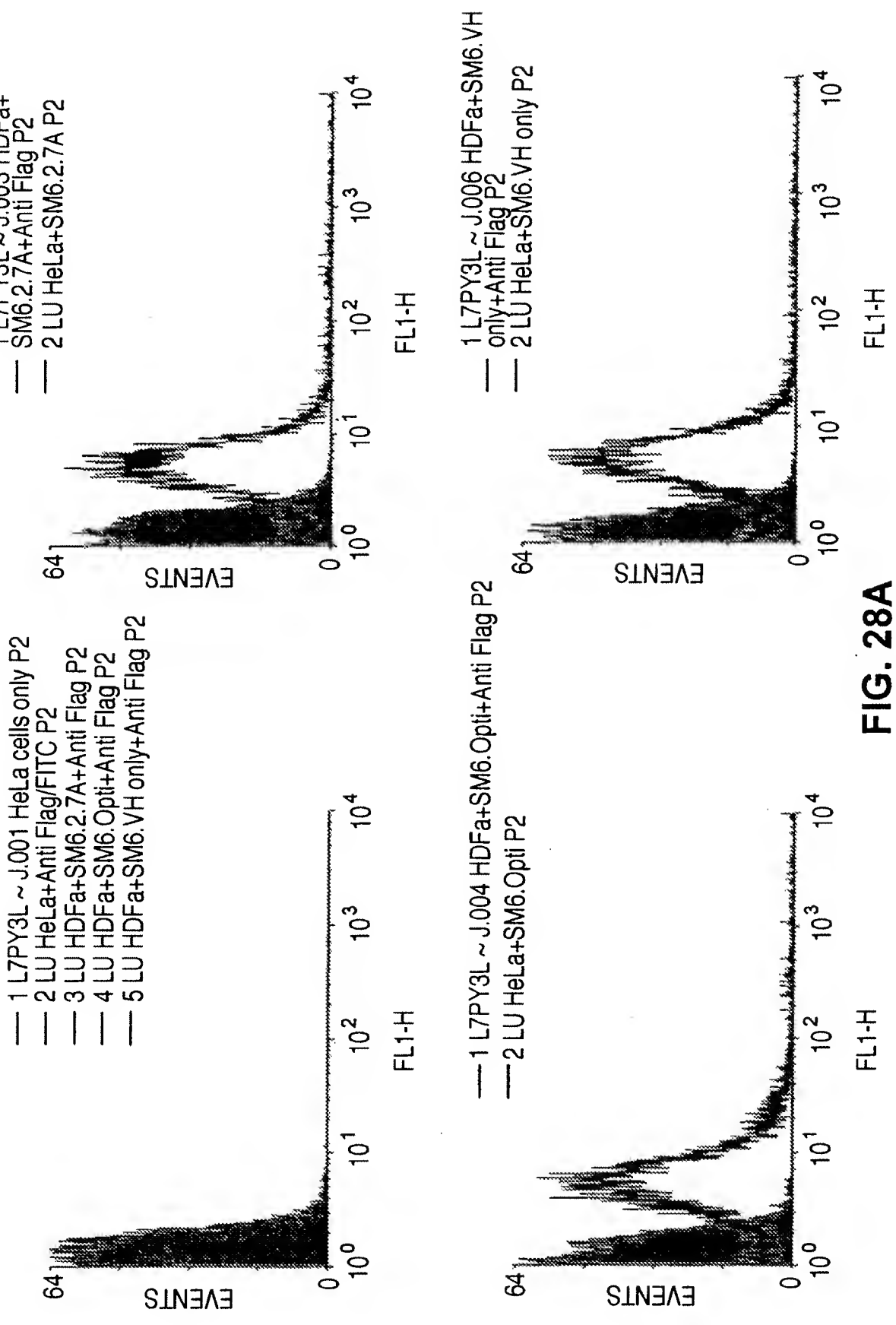

1 L7PY3L ~ J.003 HDFa+ SM6.2.7A+Anti Flag P2
2 LU HeLa+SM6.2.7A P2
EVENTS
FL1-H
1 L7PY3L ~ J.001 HeLa cells only P2
2 LU HeLa+Anti Flag/FITC P2
3 LU HDFa+SM6.2.7A+Anti Flag P2
4 LU HDFa+SM6.Opti+Anti Flag P2
5 LU HDFa+SM6.VH only+Anti Flag P2
EVENTS
FL1-H
1 L7PY3L ~ J.006 HDFa+SM6.VH only+Anti Flag P2
2 LU HeLa+SM6.VH only P2
EVENTS
FL1-H
1 L7PY3L ~ J.004 HDFa+SM6.Opti+Anti Flag P2
2 LU HeLa+SM6.Opti P2
EVENTS
FL1-H

FIG. 28A

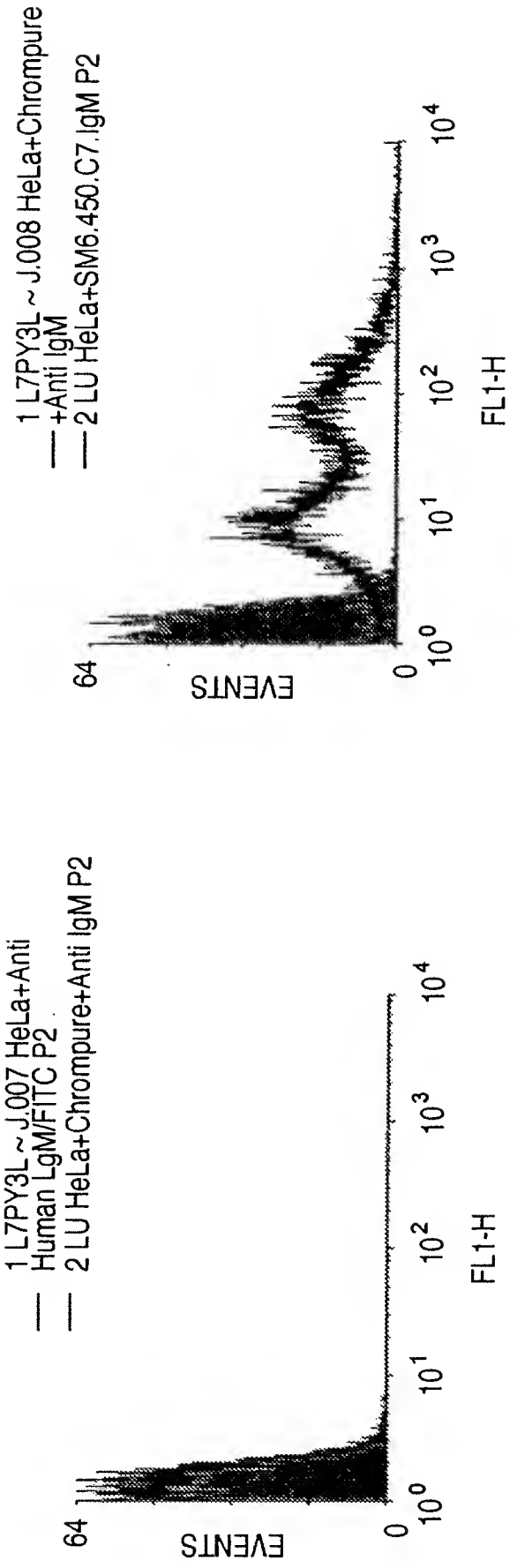

1 L7PY3L ~ J.008 HeLa+Chrompure
+Anti IgM
2 LU HeLa+SM6.450.C7.IgM P2
64
EVENTS
0
10^0
10^1
10^2
10^3
10^4
FL1-H
1 L7PY3L ~ J.007 HeLa+Anti
Human LgM/FITC P2
2 LU HeLa+Chrompure+Anti IgM P2
64
EVENTS
0
10^0
10^1
10^2
10^3
10^4
FL1-H

FIG. 28B

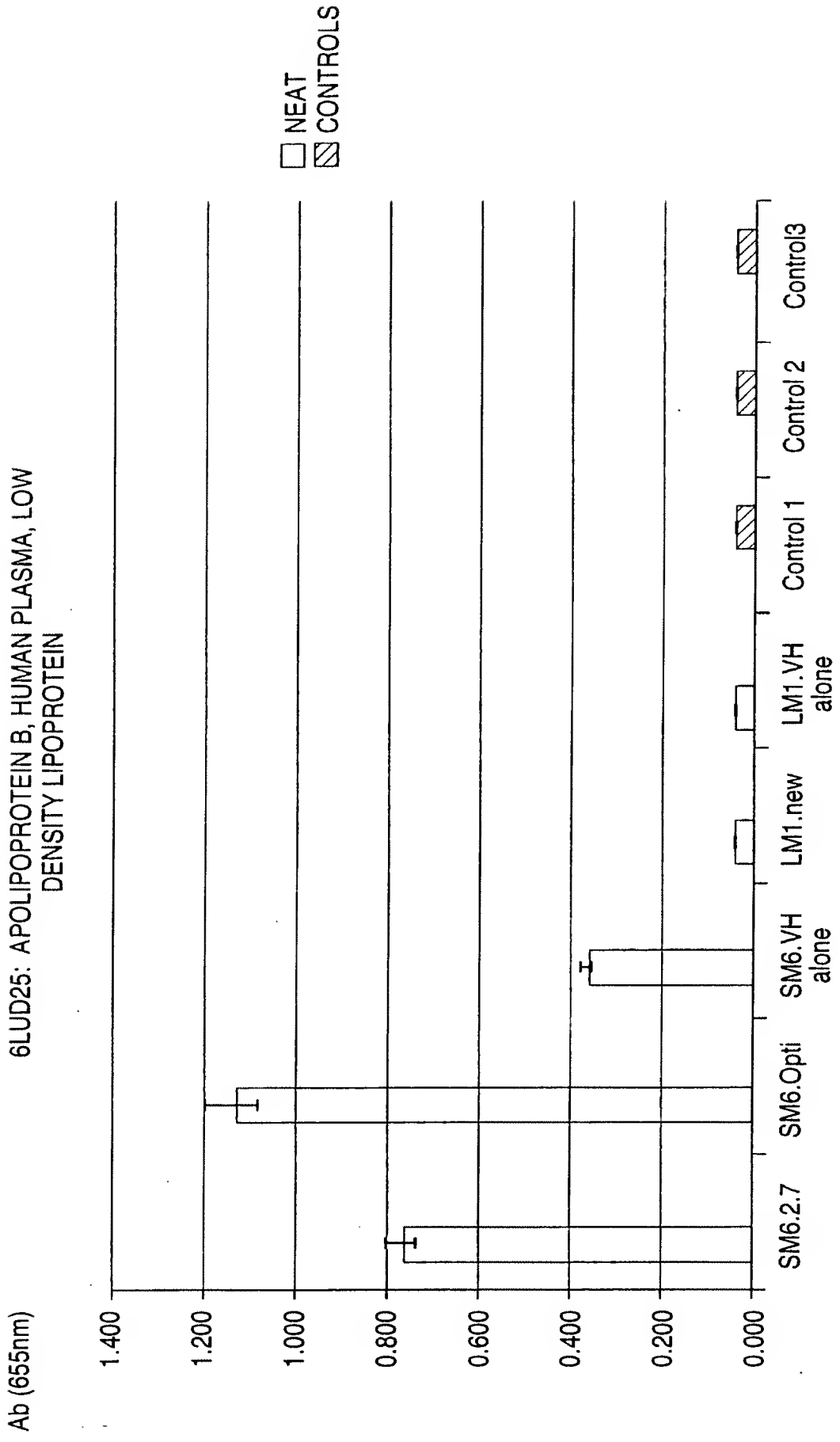

6LUD25: APOLIPOPROTEIN B, HUMAN PLASMA, LOW DENSITY LIPOPROTEIN
NEAT
CONTROLS
Ab (655nm)
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
SM6.2.7
SM6.Opti
SM6.VH alone
LM1.new
LM1.VH alone
Control 1
Control 2
Control3

FIG. 29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2010/000128

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C07K 16/30 (2006.01) *A61K 39/395* (2006.01) *A61P 35/00* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GenomeQuest: protein databases – SEQ ID NOs: 13, 15, 17 and 18
STN: Registry, CAplus – CDR sequences
STN: CA, Medline, Biosis, WPIDS – keywords (sam-6 and like terms)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/047332 A1 (ONCOMAB GMBH) 26 May 2005 Abstract, pages 34-36, claims, SEQ ID Nos: 1-4	1-6, 8, 10-54, 63-92 and 94-102
X	US 2008/0199475 A1 (VOLLMERS) 21 August 2008 SEQ ID Nos: 13-16, paragraphs [0004], [0011], [0017] and[0107]-[0112]	1-92 and 94-102
X	US 2008/0045701 A1 (VOLLMERS, P.) 21 February 2008 Abstract, SEQ ID Nos: 1-4 and paragraphs [0030] and [0034] to [0035]	1-6, 8, 10-54, 63, 93 and 100-102
P,X	WO 2009/104100 A2 (PATRYS LIMITED) 27 August 2009 paragraphs [0031], [0012]-[0014], [0033] and [0053]-[0055], SEQ ID NO:23 on page 32, SEQ ID NO:14 on page 31, SEQ ID NO:11 on page 29	1-92 and 94-102

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2010	**08 APR 2010 (08.04.2010)**
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **SONITA SINGH** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2658

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2005/047332	AU	2004289821	CA	2545454	EP	1531162
		EP	1709083	US	2008108133		
US	2008199475	AU	2007355108	CA	2683287	EP	2101813
		WO	2008/152446				
US	2008045701	DE	10353175	EP	1682580	WO	2005/049635
WO	2009/104100	US	2009202570	US	2009258020	US	2009291083
		WO	2009/080753	WO	2009/087173	WO	2009/087577
		WO	2010/004438				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX